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# BLOOD ELECTROLYTE STUDIES DURING HISTAMINE SHOCK IN DOGS

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These experiments were undertaken in order to study the changes occurring in the acid-base equilibrium during acute shock. The injection of histamine into an animal causes capillary dilatation, with drop in blood pressure and stasis not unlike the picture of shock as it occurs complicating the course of various clinical conditions. A response so easily produced and readily controlled seemed eminently suitable for a physicochemical study of shock.

Dale and Laidlaw (1) showed that, during the course of histamine shock there was a concentration of the circulating blood, in the sense that the ratio of corpuscle volume to serum volume was markedly increased. Inasmuch as the primary effect of histamine had been found to be capillary dilatation, they inferred that this concentration was due chiefly to transudation of serum through the capillary walls. The scope of their investigations did not include any observations on the physicochemical changes in the blood. In a later study, they also found that there was a corresponding decrease in plasma volume, and that the protein content of the serum during histamine shock remained essentially unchanged, and their conclusion was that whole serum escaped through the capillary walls. Lewis (2) offered indirect evidence in favor of the same conclusion by finding that the proteins of a histamine wheal (skin) closely approximated those of blood serum.

The observations here presented were made immediately before and during histamine shock in dogs and include serum electrolyte partitions, hematocrit determinations and in certain instances  $O_2$  capacity and serum pH.

TABLE I

Blood electrolytes during histamine shock

Date	D <sub>2</sub> -D <sub>2</sub> -D <sub>2</sub>	Mg	K <sub>2</sub> mm	Na <sub>2</sub> per 100 cc	Sodium bicarbonate mEq/100 cc	Sodium pro- tein mEq/100 cc	Total bicar- bonate mEq/100 cc	Total acid mEq/100 cc	Total base mEq/100 cc	Total base and acid mEq/100 cc	Pota- ssium mEq/100 cc	Chloride mEq/100 cc	Carbonate mEq/100 cc	Protein mEq/100 cc	Phosphate mEq/100 cc	Lac- tate mEq/100 cc	CO <sub>2</sub> tension mm	pH	Serum			Hartman		
January 30, 1929	1	M	10.0	39	5.7	153.2	150.1	2.8	111.8	23.4	1.7	13.5					11.2	10.15	5 mgm at					
				39	5.7	156.2	119.3	6.9	111.4	22.3	2.1	13.5					53.3	10.25	10.20					
January 30, 1929	2	M	9.0	39	6.0	161.9	117.2	14.7	111.6	19.8	1.6	14.2					48.6	10.35						
				39	6.3	152.7	148.1	4.3	103.6	27.1	2.8	14.9					53.0	11.51	11.45					
February 6, 1929	3	I	8.7	34	5.8	156.3	152.3	4.0	114.8	21.8	2.0	13.7					56.0	11.23	10.57-					
				34	5.8	156.0	116.2	9.8	112.2	17.9	2.4	13.7					11.04	11.16	11.13					
February 6, 1929	4	I	8.3	21	5.6	155.7	150.2	5.5	111.6	23.1	2.3	13.2					38.5							
				21	5.9	160.6	119.1	11.5	112.6	19.0	3.6	13.9					46.0	2.19-	2.13					
February 27, 1929	6	M	11.3	29	5.3	155.1	149.2	5.9	114.8	20.1	1.7	12.6					47.0	10.22	10.45	10.45	1.13			
				29	5.4	158.0	116.5	11.5	114.0	17.6	2.1	12.8					112.0	22.3	2.6	11.22				
				5.2	157.9	119.1	8.8										42.4							

April 4	1929	8	F	15.4	{	28	6.4	159.5	151.6	7.9	111.4	22.8	2.2	15.2		37.5	18.2	10.5	10.27	10.14		
							6.1	163.4	146.3	17.1	111.4	16.5	3.9	14.5		50.0	23.7	10.32	10.20			
May 16,	1929	9	M	8.1	{	25	6.5	153.3	153.7	-0.4	109.6	23.4	1.6	15.4	3.7	40.0	9.50	7.0	mgm. at			
							6.1	162.0	163.9	-1.9	111.4	16.0	3.1	14.3	19.1	46.5			10.32	10.20		
June 6,	1929	10	M.	19.4	{	28	6.8	158.9	152.2	6.7	110.0	21.5	1.9	16.2	2.6	41.0	10.00	10.00	10.00	10.14		
							6.8	162.3	160.3	2.0	110.6	16.6	2.9	16.2	14.1	52.5			10.25			
March 14		11	F	15.0	{	32	7.0	157.1	153.8	3.3	23.7	109.8	21.6	2.1	16.5	3.8	40.07	36.39	3.19	4.74	9.20	
1930							3.6	7.3	161.6	157.6	4.0	23.1	108.4	17.6	2.8	15.5	13.3	49.07	15.58	0.26	4.42	9.53
																				9.33		
September		14	F	19.0	{	36	6.4	159.9	153.0	5.9	103.8	26.7	3.0	15.0	4.5	59.57	27.40	7.20	6.76	10.41		
16 1930							6.8	162.1	157.5	4.6	99.9	24.2	4.1	16.0	13.3	77.07	10.53	5.25	4.24	11.29		
							6.0	161.8	155.9	5.9	99.2	22.8	4.4	14.2	15.3	49.622	6.33	11.46				

\* See paper for description of histamine administration.

## METHODS

Dogs were used in all experiments. The animal was strapped on the operating board, and 80 cc of blood drawn under oil from the external jugular vein, the dogs nearly always submitted to this procedure quietly. A solution of histamine acid phosphate containing 5 to 12 mgm of histamine base was then injected rapidly into 1 leg vein. The usual reaction to this was a certain amount of convulsive struggling lasting about half a minute, associated with defecation, salivation and urination. The pulse became thready or imperceptible, a deep red flush gradually appeared over the skin, turning in the course of a minute or two to a deep cyanosis. At the height of this latter effect, another 80 cc sample of blood was drawn from the jugular vein. In certain of the animals a third sample was drawn at a later period (see table 1).

In the last experiment (dog 14), the animal was anesthetized, and a considerable variation in technique employed. The special purpose of this experiment was to discover whether the transient convulsive response to histamine, in the conscious animal, had been an important factor in the changes we had found. This dog was first anesthetized rapidly with open ether, then injected with 5 grams of sodium veronal intravenously. Deep anesthesia was produced, inspirations were greatly decreased for the next half hour, but subsequently increased again. The dog was left undisturbed for the first 2 hours after veronal injection. At the end of this time, the animal was completely relaxed, breathing slowly and evenly, and the heart action was apparently normal. The carotid artery was exposed, cannulated, and connected with a mercury manometer for the registration of blood pressure (Glucose solution was used in the connecting tubing, to prevent electrolytes entering the circulation). Jugular and femoral veins were exposed to facilitate taking of blood samples and injection of histamine. After a preliminary blood sample had been drawn, histamine injections were begun, and given almost continuously, at the rate of 0.1 to 0.5 mgm every 1 to 2 minutes, for the next hour. During the last 40 minutes of this time, the arterial blood pressure remained between 35 and 40 mm. Blood was drawn 40 minutes after the start of the histamine injections, and again 20 minutes later. No convulsive movements of any kind were noted during the course of these injections.

The chemical methods have, with the few exceptions noted below, been discussed in a previous paper (3). Lactic acid was determined by the method of Friedemann, and Kendall (4). Blood gas analyses were performed with the Van Slyke-Neill (5) and Haldane apparatus, the technique of blood manipulation being essentially that of Austin, Van Slyke et al (6). For the determination of serum pH, CO<sub>2</sub> dissociation curves of whole blood were constructed, the calculations were made with the aid of the Van Slyke-Sendroy (7) charts. In calculating base bound to protein, Van Slyke's formula for human serum was used, as none is available for dogs' blood. Hematocrits were done on samples containing equal and constant amounts of oxalate.

## RESULTS AND DISCUSSION

The results of the experiments are presented in table 1. It is obvious from the changes in hematocrit and (when measured) O<sub>2</sub> capacity that a striking concentration of the blood occurs during histamine shock. The greatest increase was 18.7 per cent (by hematocrit reading) in Dog 11, and in no instance was it absent. Furthermore, it appears clear that this was not a concentration by loss of water, for the protein percentage of the serum changed very little, if any, in most of the animals. In such a brief experiment the serum protein concentration is a fairly reliable index of dilution by water alone. The concentration of the blood during histamine shock seems, therefore, to be dependent upon the transudation of whole serum through the capillary walls, as shown previously by Dale and Laidlaw.

As the work progressed it was observed that there was always an increase in the undetermined acid of the serum, i.e., total base minus total acid (B-A), during the period of shock. As shock causes striking vascular stasis in the tissues with probably significant interference with their oxidative processes, it was suspected that this undetermined acid was lactic acid. Lactate determinations on the last four animals proved this assumption to be correct. As much as 15 m eq of lactate were found in one of the dogs. The sudden, brief twitching that occurred with the injection of histamine in the conscious animal seemed insufficient to cause the lactic acid acidosis, and the experiment on Dog 14, performed under veronal anesthesia, indicates that excessive lactic acid production occurs in the absence of any convulsive muscular movements. The occurrence of lactic acid acidosis is, therefore, a constant accompaniment of histamine shock and is probably due to tissue anoxemia.

Another constant change during histamine shock was a considerable percentage increase in serum phosphate. No satisfactory explanation of this finding is apparent from these experiments.

An interesting and somewhat unexpected observation was the effect of histamine shock on the total base of the serum. In every instance there was an increase in total base, and in 6 out of 10 dogs it was well outside the limits of error of the method. When the lactic acid increase was large, there was always a significant increase in base. In a

fairly large series of total base determinations on animals and human beings under a variety of conditions, we have never seen increases in serum total base independent of base administration. It is the more noteworthy, therefore, that the only instance of consistent base increase to be found in the literature was reported by Henderson and Bock (8) following the lactic acid acidosis of muscular fatigue. The acidosis of histamine shock and muscular exercise are similar not only in the fact that lactic acid is the common cause, but also by virtue of the fact that they both occur rapidly. It is impossible to determine from our data the relative importance of these two factors in the production of the unusual compensatory mechanism evidenced by an increase in serum cations. In dog 11, potassium determinations were made in order to throw light on the source of the increased base. No change in potassium was found which leads one to infer that the base increase was probably not drawn from the cells.

Serum bicarbonate decreased consistently as base was needed for lactate, for the increase in total base was less than the lactate production. It should be noted that a slower induction of shock (and thus acidosis) in the anesthetized animal was accompanied by a decrease in chloride greater than that of bicarbonate. In the other animals chloride concentrations were all surprisingly constant. There was a considerable decrease in serum pH and increase in  $\text{CO}_2$  tension in the three cases measured. As would be expected,  $\text{O}_2$  saturation decreased markedly in the four animals in which that determination was included.

#### CONCLUSIONS

Acute histamine shock in dogs is accompanied by

1 Concentration of the blood due to the transudation of whole serum. This is proved by the striking change in hematocrit and  $\text{O}_2$  capacity with no significant accompanying change in serum protein.

2 Uncompensated lactic acid acidosis, with the required base afforded by (a) increase in serum total base, (b) decrease in serum bicarbonate. One experiment performed under veronal anesthesia was sufficiently similar to the other experiments to suggest that this formation of lactic acid was not due to muscular contraction.

3 Increase in inorganic serum phosphate unexplained by these experiments.

4 Decrease in  $\text{O}_2$  saturation.

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## STUDIES ON GALLBLADDER FUNCTION

### II. THE ABSORPTION OF SODIUM TETRAIODOPHENOLPHTHALEIN FROM THE GALLBLADDER

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Although many data have been presented in the past few years which indicate that the gallbladder empties its contents through the cystic duct, there are those who believe that removal of the contents of the gallbladder is brought about entirely by an absorptive mechanism (Blond (1), Sweet (2), Demel and Brummelkamp (3), and Halpert (4)). Recently several of the workers who do not accept the contractile theory (1, 2) of gallbladder evacuation, have maintained that the disappearance of the gallbladder shadow in cholecystography is dependent on absorption from the gallbladder rather than the passage of the dye through the cystic duct.

The hypothesis presented by these workers (1, 2) should not be a difficult one to prove or disprove by experimental methods. If the hypothesis is correct in whole or in part, there should be (1) a gradual disappearance of the shadow whether the egress of bile from the cystic duct were prevented or not, and even though the cystic duct be occluded (2) a greater rapidity in the disappearance of the shadow following a fat meal, and (3) a rapid decrease in the amount of dye in the gallbladder as estimated by analytical methods. It was to test these criteria that these studies have been made.

#### METHOD

The experiments in this report were carried out on dogs. The technique employed was similar to that used by workers who have attempted to drain separate lobes of the liver. In the main we have

<sup>1</sup> Harriett M. Frazier Fellow in Surgery

followed the technique of Sweet. With this technique advantage is taken of the fact that in the dog the right lower lobe duct enters the common duct a short distance from the entrance of the common duct into the duodenum (fig 1). Animals fasted for 12 hours were operated upon under sodium amytal anesthesia. A ligature was placed on the common duct and left hepatic duct above the point of entrance of the right lower lobe duct. The common duct was cannulated below the



FIG 1 METHOD OF CANNULIZATION WHICH PERMITS COLLECTION OF BILE FROM  
RIGHT LOWER LOBE DUCT

entrance of the right lower lobe duct and the bile from this was allowed to flow into a rubber bag left inside the abdomen. Material from the gallbladder cannot find its way into the bag directly, but in order to reach the bag must pass into and be excreted by the right lower lobe of the liver after being absorbed from the gallbladder, or find its way into the liver through an open duct which connects the liver and gallbladder. Sodium tetraiodophenolphthalein or potassium iodide was introduced into the gallbladder by puncturing the gall-

bladder wall with a small bore needle, removing the bile contained within, and introducing the salt before removing the needle. The puncture hole was tied off by a purse string suture. The animals were sacrificed at the end of 24 hours and the contents of the gallbladder and collecting bag removed, to be analyzed for iodine. Each animal was x-rayed immediately after the operation and just before being sacrificed.

The method of Fresenius (5) for iodine assay was used for the most part, except in a few instances when iodine was determined by the method of Kendall (6) for purposes of comparison. Either of these methods is suitable, the Fresenius method being, in our experience, simpler and more dependable when determining as large quantities as were used in these experiments. With either method, the material was fused with sodium hydroxide and sodium nitrate as described by Kendall (6).

#### RESULTS

In the first group of animals the ducts were prepared as described and a known amount of potassium iodide was placed in the gallbladder. The animals were not fed during the succeeding 24 hours. Reference to table 1 shows that a major portion of the potassium iodide left the gallbladder during the course of the experiment and a large portion of this was recovered from the collecting bag draining the right lower lobe of the liver.

In a second group of animals known amounts of sodium tetraiodophenolphthalein were introduced into the gallbladder with a similar technique (table 2). In these experiments we found that iodine did not leave the gallbladder as rapidly as when potassium iodide was used. Twenty-four hours after the introduction of the potassium iodide 2.7 to 6.7 per cent of the original amount was recovered from the gallbladder. In the experiments with sodium tetraiodophenolphthalein from 36.9 to 55.8 per cent was recovered from the gallbladder.

These experiments with potassium iodide and sodium tetraiodophenolphthalein were performed during a period of fasting. It was deemed advisable to repeat the sodium tetraiodophenolphthalein experiments and to observe the effect of a fat meal on the disappearance of the dye from the gallbladder.

Three animals were given 100 cc of cream six hours before being killed. In these animals from 12.3 to 21.4 per cent of the iodine was recovered from the gallbladder. These percentages (table 3) approach those obtained from fasting animals with the use of potassium iodide.

Since the animals studied in Groups I, II, and III had an open cystic duct which would permit of direct communication with the

TABLE 1

*Recovery of iodine from gallbladder and right hepatic duct following introduction of potassium iodide*

Dog	Date	Amount of salt placed in gallbladder	Amount of iodine placed in gallbladder	Amount of iodine recovered from gallbladder	Amount of iodine recovered from bag	Percentage iodine recovered from gallbladder
	1930	grams	grams	grams	grams	
9	January 21	0.925	0.707	0.019	0.098	2.7
17	February 14	0.414	0.316	0.020	0.087	6.3
20	February 22	0.411	0.314	0.021	No collection	6.7
21	February 22	0.400	0.306	0.010	0.093	3.3

TABLE 2

*Recovery of iodine from gallbladder and right hepatic duct following introduction of sodium tetrachlorophenolphthalein*

Dog	Date	Amount of salt placed in gallbladder	Amount of iodine placed in gallbladder	Amount of iodine recovered from gallbladder	Amount of iodine recovered from bag	Percentage iodine recovered from gallbladder
	1930	grams	grams	grams	grams	
15	February 7	0.500	0.300	0.156	0.021	52.0
27	March 27	0.542	0.325	0.120	0.054	36.9
28	March 27	0.418	0.251	0.140	0.031	55.8

Cystic and accessory ducts not occluded

No food given animals

liver a fourth group of animals, whose cystic ducts were occluded, was studied. In order to ascertain whether feeding affected the rate of absorption of the iodine in this group some of the animals were given 100 cc of cream 6 hours before being sacrificed while others were fasted during the entire experiment.

The occlusion of the cystic duct was obtained in some animals by a ligature which excluded the cystic blood vessels. In order to be

certain that the ligature was not influencing the results by obstructing lymph vessels, the ducts, in other animals were not tied, but were occluded by filling the lumen of the common and hepatic ducts above the ligature with low melting point paraffin. The results by either

TABLE 3

*Recovery of iodine from gallbladder and right hepatic duct following introduction of sodium tetraiodophenolphthalein\**

Dog	Date	Amount of salt placed in gallbladder	Amount of iodine placed in gallbladder	Amount of iodine recovered from gallbladder	Amount of iodine recovered from bag	Percentage iodine recovered from gallbladder
16	February 7	0.500 grams	0.300 grams	0.037 grams	0.120 grams	12.3
37	May 2	0.500	0.300	0.049	0.092	16.3
38	May 2	0.560	0.336	0.072	No collection	21.4

\* 100 cc. cream given on day following operation

Cystic and accessory ducts not occluded.

TABLE 4

*Recovery of iodine from gallbladder and right hepatic duct following introduction of sodium tetraiodophenolphthalein*

Dog	Date	Amount of salt placed in gallbladder	Amount of iodine placed in gallbladder	Amount of iodine recovered from gallbladder	Amount of iodine recovered from bag	Percentage iodine recovered from gallbladder
39	May 7	0.497 grams	0.298 grams	0.152 grams	No collection	51.0*
40	May 8	0.195	0.117	0.031	0.025	26.5*
41	May 12	0.507	0.304	0.111	0.030	36.5*
45	June 4	0.509	0.305	0.100	0.012	32.8*
42	May 19	0.500	0.300	0.090	No collection	30.0†
43	June 2	0.490	0.294	0.081	0.025	27.6†

Cystic duct occluded

\* 100 cc. cream given on day following operation

† No food given animal.

method were identical. From 26.5 to 51.0 per cent of the iodine was recovered from the gallbladder at the conclusion of the experiments (table 4). The results are similar to those obtained from fasting animals without occlusion of the cystic duct.

In the experiments in which sodium tetraiodophenolphthalein was

placed in the gallbladder there was, at autopsy, a coating of blue material upon the walls. This proved to be concentrated salt and mucus and required careful scraping and washing to remove it. The dye remaining on the mucosa contained most of the iodine remaining in the gallbladder and if this were not taken into account the apparent disappearance of the material would be increased to a great extent.

#### DISCUSSION

It is of definite importance in clinical cholecystography to know whether the disappearance of the shadow is the result of absorption of the dye through the gallbladder wall, or whether it is the result of a contractile mechanism which empties the gallbladder. Sweet (2), from a study which was in certain respects similar to this one, in that he obtained his data from experiments comparable to the experiments in Group II, concluded that the changes in the gallbladder shadow in cholecystography should be interpreted as the result of absorption. This would confirm his statement that he has not found it necessary to change his conclusions expressed in 1923 that, "under normal conditions, whatever passes into the gallbladder through the cystic duct never passes out again through the cystic duct." Halpert, whose early experiments (7) with methylene blue had led him to similar conclusions, has since repeated his experiments (8) and found it necessary to alter his previous conception.

The experiments reported in table 1 show that potassium iodide is rapidly absorbed from the gallbladder. However, it is not logical to deduce that a complex salt such as sodium tetraiodophenolphthalein will do likewise. In the experiments reported in table 2 where the latter salt is used under similar conditions the iodine remains in the gallbladder in greater amounts over the same period of time.

There is, however, a serious defect in the planning of this experiment. The cystic and central hepatic ducts are patent, as are the accessory ducts emptying into the cystic duct so that a direct communication exists between the gallbladder and the liver. It was because of this that the experiments reported in tables 3 and 4 were planned. In the experiments reported in table 3 the animals were given a fat meal six hours before being sacrificed. The disappearance of a greater amount of iodine can be interpreted in one of two ways:

It could be argued that the fat meal increased absorption. On the other hand, the proponents of the contractile theory of gallbladder evacuation might say that it forced the dye into the hepatic ducts, to the liver, and was then excreted through channels which were not occluded.

The experiments reported in table 4 were done on animals whose cystic and accessory ducts were occluded, as was proven at autopsy. Some received a fat meal while others were fasted. In these experiments the amount of iodine recovered from the gallbladder closely simulates that recovered from the fasting dog whose cystic duct was not occluded. In the light of these experiments it is impossible to draw the conclusion that a fat meal increases absorption from the gallbladder under the conditions of these experiments.



FIG 2 ROENTGENOGRAMS OF GALLBLADDER OF DOG 38 TAKEN, (A) IMMEDIATELY AFTER THE INJECTION OF SODIUM TETRAIODOPHENOLPHTHALEIN INTO THE GALLBLADDER, AND (B) 24 HOURS AFTER THE INJECTION

An air bubble is seen in A, bile ducts are outlined in both pictures. B shows a well outlined periphery.

On the other hand, roentgenograms taken in these experiments indicate that the dye does escape from a patent cystic duct when a fat meal is given (fig 2). The use of roentgenograms for the determination of the quantity of iodine remaining in the gallbladder proved to be unreliable. This is to be expected upon theoretical grounds, as there are many variables to be considered, such as the size of the animal, the concentration of the salt within the gallbladder and the size of the gallbladder. The last two of these factors will be affected by variation in the rate of water absorption from the gallbladder. While it is true that in all our experiments there was a decrease in the density of the gallbladder shadow, the lessened density was not always proportional to the decrease of iodine within the gallbladder.

Sweet (2) contends that in the human subject the gallbladder shadow becomes smaller because the iodine is absorbed from around the walls of the gallbladder so that the concentration of iodine becomes lessened at the periphery and the shadow is cast by the concentrated dye remaining in the center of the gallbladder. When one considers that the gallbladder is kept in motion by respiratory movements, it is



FIG 3 ROENTGENOGRAMS OF GALLBLADDER OF DOG 10, AN EXPERIMENT SIMILAR TO THOSE IN TABLE 2

*A* was taken immediately after the introduction of sodium tetraiodophenolphthalein, *B* and *C* were taken 24 hours later. *C* was taken after the removal of the gallbladder. Note the moth eaten appearance of the shadow cast by the gallbladder in *B* and *C*, due to the precipitation of the salt. The periphery is well outlined in (*B*)

difficult to understand how the concentration of iodine could vary considerably in different portions of the lumen of the gallbladder. Even though sodium tetraiodophenolphthalein were not freely diffusible in solution, physical mixing would tend to keep the solution within the gallbladder practically homogeneous.

In none of our roentgenograms was there any evidence of increased density in the center of the shadow. On the contrary, there was

quite uniformly an increased density at the periphery of the shadow in the 18 hour roentgenograms (figs 1 and 2)

Our data with sodium iodide are in agreement with the findings of Sweet. Sodium tetraiodophenolphthalein, however, was found to leave the gallbladder much more slowly so that we constantly recovered more from the gallbladder at the conclusion of an experiment than we did from the bag draining the right lower lobe except when the cystic duct was patent and a fat meal was given the animal.

I wish to thank Dr I S Ravdin for advice and for his interest in this problem and Dr H K Pancoast and Dr E P Pendergrass for the interpretation of the roentgenograms.

#### SUMMARY

- 1 Potassium iodide is rapidly absorbed from the gallbladder
- 2 Sodium tetraiodophenolphthalein is absorbed more slowly than potassium iodide
- 3 The rate of absorption of sodium tetraiodophenolphthalein is so slow that it does not seem possible for absorption to explain the rapid changes occurring in clinical cholecystography
- 4 We have not obtained any evidence which suggests that a fat meal increases the rate of absorption of the dye

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# RESPONSES TO THE INJECTION OF EPINEPHRINE IN HEPATIC DISEASE

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## INTRODUCTION

The important rôle of the liver in carbohydrate metabolism has been repeatedly emphasized in recent years, and particular attention has been paid to its function in the maintenance of the blood sugar level and the storage of glycogen (1, 2).

Minkowski (3), in 1886, observed the hypoglycemia which developed in geese following hepatectomy and also noted, in the same experiments, a great increase in the urinary excretion of lactic acid. In 1912, Parnas and Baer (4) demonstrated the rôle of the liver in synthesizing glycogen from lactic acid. In 1906, von Noorden and Embden (5) suggested the existence of a "chemical carbohydrate cycle" in which liver glycogen is converted into glucose, which in turn is polymerized in the muscles to glycogen, which then yields lactic acid, and the latter is transported back to the liver to be converted again into carbohydrate. Barr, Himwich, and Green (6) in 1923 demonstrated that lactic acid liberated from leg muscles during exercise is temporarily stored in resting muscles elsewhere. Janssen and Jost, (7), found that during the infusion of d lactic acid, this substance is removed by the muscles, but with the discontinuation of the infusion, enormous quantities of lactic acid are poured into the venous blood from the muscles and are then presumably synthesized into glycogen by the liver. In the past three years, the carefully controlled experiments of Cori and Cori (9) on the mechanism of the action of epinephrine have yielded quantitative evidence supporting the idea of this carbohydrate cycle and have demonstrated the importance of the liver in the cycle. These authors have pointed out that epinephrine decreases carbohydrate utilization in the muscles, increases the mobilization of muscle glycogen, thus augmenting lactic acid formation, which subsequently increases the glycogen storage in the liver. They have also pointed out that epinephrine hyperglycemia results from an initial but transient glycogenolysis in the liver and that the hyperglycemia is then maintained by the decreased glucose utilization on the part of the muscles.

On the basis of the work described, numerous clinical studies have been made on the behavior of the lactic acid and of the blood sugar in the presence of liver disease (10-20). In general it has been shown that (a) the lactic acid content of the blood is increased in patients with severe liver disease, (b) there is a delay in the disappearance of sodium lactate injected intravenously, (c) feeding of sodium lactate occasionally raises the blood lactic acid level more in patients with liver disease than in normal individuals, (d) the hyperglycemia following the injection of epinephrine is less marked in cases of hepatic insufficiency than in those with normal livers, probably because of decreased glycogen stores.

In 1923, Tolstoi et al (8) found an increase in the lactic acid content of the blood of four normal individuals following the injection of 15 minimis of adrenalin. Cori and Cori (9) have recently demonstrated that following the injection of epinephrine in normal animals, there is not only prolonged elevation of the blood sugar but that the lactic acid content of the blood is also increased for some hours. They have observed, furthermore, that the rise in concentration of lactic acid in the venous blood is considerably greater than that in arterial blood. This result is to be expected in view of experiments showing increased glycogen storage in the liver one or more hours after the injection of epinephrine.

From the foregoing discussion it is apparent that the injection of epinephrine causes a prolonged out-pouring of lactic acid from the muscles, which is normally removed by the liver and resynthesized to glycogen. It seemed possible that this mechanism might be interfered with in patients with disease of the liver, and that there would result a greater accumulation of lactic acid in the blood than would be found in normal individuals. For this reason we have studied the blood lactic acid curves following the subcutaneous injection of adrenalin, and simultaneously the changes in blood sugar and blood pressure have been observed.

#### EXPERIMENTAL

The studies were made on human subjects in the fasting state and at rest in bed. The "normal" group of controls, except for one medical

student who was ambulatory, consisted of ward patients in whom no pathological condition could be demonstrated. The cases of jaundice investigated included the following groups of conditions: (a) intrahepatic jaundice including catarrhal jaundice, toxic hepatitis and acute yellow atrophy of the liver, (b) obstructive jaundice due to carcinoma of the pancreas or bile ducts, (c) cirrhosis of the liver, (d) cholecystitis with cholelithiasis. Observations were also made on a group of miscellaneous disease conditions and on six patients suffering from diabetes mellitus.

Blood was withdrawn from an arm vein without stasis and the blood pressure was measured. Following this, 0.7 to 1.0 cc. of epinephrine (Armour and Co.) was injected subcutaneously. The blood pressure was determined at intervals of 2 to 3 minutes for one-half hour and at intervals of about 5 minutes during the next thirty minutes. Samples of venous blood were removed one-half, one, and frequently two hours after the adrenalin injection. Blood samples were treated with sodium fluoride and were analyzed without delay for glucose and lactic acid. In the earlier experiments, sugar alone was determined. In later studies blood was not taken two hours after epinephrine injection, because experience demonstrated that the maximum concentrations of sugar and lactic acid were almost invariably reached in one hour.

In a few instances lactic acid was determined in the urine excreted during the two hours following the administration of adrenalin, but as there was no increase over the normal amount, this procedure was discontinued. On the basis of the experiments of Jervell (14) and of Hewlett, Barnett and Lewis (21), no increase in urinary excretion of lactic acid was to be expected.

Lactic acid was determined by the method of Friedemann and Kendall (22) modified in some of its details. The procedure gave excellent results with pure lactate solutions, but was not reliable within  $\pm 5$  per cent when applied to blood or urine samples. The determinations were made on protein-free filtrates of whole blood. The method of Fohn and Wu (23) was used for blood sugar measurements. All analyses were made in duplicate. The lactic acid and the glucose of the blood have been recorded in terms of millimols per liter so that comparative changes in molar concentrations might be observed.

Effect of epinephrine in normal

Hospital number	Sex	Age	Diagnosis	Dose of epi neph rine	Blood sugar						Blood sugar increase	
					Initial	Time after epinephrine						
						½ hour	1 hour	2 hours				
				cc	mills molts per liter	per cent						
219386	M	20	Normal	1 0	5 27	7 66	8 66	6 22	3 39	65		
	M	24	Normal	1 3	4 66	6 83	9 11	8 66	4 45	96		
239481	F	25	Neurosis	1 0	5 56	8 72	9 40	7 39	3 84	69		
6093	F	48	Neurosis	1 0	5 66	9 38	10 6		4 94	87		
240839	F	17	Hysteria	1 0	5 00	8 17	9 06		4 06	81		
235105	F	34	Healed lung abscess	1 0	5 67	8 22	9 84	9 40	4 17	74		
<b>Average</b>					5 30	8 16	9 45	7 92	4 16	79		
75933	M	60	Hypertension myocar- ditis	1 0	6 16	6 00	6 61	6 61	0 45	7		
132607	F	41	Picks disease	1 0	5 33	6 23	7 28	6 55	1 95	37		
241332	M	28	Tuberculous peritonitis	1 0	5 00	5 44	5 78		0 78	16		
75023	F	18	Debility, hookworm	0 7	5 00	7 40	6 06		2 40	48		
229520	M	25	Hookworm, incipient tuberculosis	1 0	5 00	6 78	7 50		2 50	50		
241666	M	22	Duodenal ulcer	1 0	4 83	5 94	6 83		2 00	41		
228061	M	43	Duodenal ulcer	1 0	4 33	5 05	5 78	5 22	1 45	34		
231234	F	39	Tuberculosis of skin	1 0	4 94	8 33	8 56		3 62	73		
233248	F	35	Addison's disease	0 7	4 77	7 17	6 78	5 22	2 40	50		
235030	M	32	Bronchial asthma	1 0	5 21	6 45	7 34	6 27	2 13	41		
236702	M	17	Convalescent rheumatic fever	1 0	4 94	6 27	7 00	5 94	2 06	42		
79533	F	63	Polycythemia vera	1 0	6 50	9 95	10 4	8 06	3 90	60		
81394	M	21	Convalescent pneumonia	0 7	5 50	8 06	8 45	6 28	2 95	54		
81537	M	17	Convalescent pneumonia	1 0	7 89	12 3	12 1	7 67	4 41	56		
<b>Average</b>					5 39	7 24	7 61	6 42	2 36	43		
66114	M	70	Diabetes and cardiac ins- ufficiency	1 0	9 78	9 72	9 45	10 0	0 22	2		
229110	M	38	Diabetes	1 0	5 78	6 45	6 61		0 83	14		
81185	F	22	Diabetes	1 0	14 6	15 4	17 1		2 50	17		
82430	F	34	Diabetes	1 0	4 28	5 22	8 05		3 77	88		
61945	M	18	Diabetes	0 7	14 2	14 4	14 8		0 60	4		
245567	M	42	Diabetes and hyperthy- roidism	1 0	18 5	19 2	19 5		1 00	5		
<b>Average</b>					11 2	11 7	12 6		1 49	22		

\* Patient had severe collapse 40 minutes after epinephrine    Blood pressure dropped to 60/30

† 1 millimol of glucose = 180 mgm

‡ 1 milli-equivalent of lactic acid = 90 mgm

*In miscellaneous disease conditions*

Lactic acid		Blood lactic acid increase		Blood Pressure				Remarks
After epinephrine	Initial			Maxi- mum	In crease	Time after epineph- rine		
1 hour	2 hours	m eq per liter	m eq per liter	mm Hg	mm Hg	per cent	minutes	
3.62	1.87	2.43	204	124/70	162/40	31	35	
3.30	2.93	2.01	156	128/84	156/76	22	35	Not in bed
2.08	1.43	1.21	139	114/73	132/70	16	31	
3.52		2.62	291	110/70	140/50	27	18	
2.57		1.65	183	100/70	140/?	40	17	
2.86	2.11	1.79	167	108/75	142/78	22	53	
2.99	2.09	1.95	190			26		
1.82	1.89	0.66	54	220/112	236/120	2	43	
2.00	1.46	1.09	120	108/66	130/72	20	13	
3.31		1.59	93	110/80	124/70	11	12	Temperature 101° to 102°
1.64		1.36	262	105/0	162/70	54	4	No anemia. Debilitated
2.59		1.21	88	112/55	134/53	20	27	No anemia or fever
2.48		1.30	110	105/60	112/60	7	3	Receiving Sippy diet
1.87	2.07	1.25	153	116/78	142/70	22	40	Receiving Sippy diet
1.71		1.02	148	110/72	200/108	82	15	
1.55	1.10	1.30	161	103/78	106/70	3	10	Bilateral adrenal tuberculosus autopsy
1.43	1.12	0.73	104	110/80	134/84	22	58	
2.33	1.39	1.43	159	102/62	128/70	25	37	Lactic acid excreted in urine · mgm
				140/92	196/70	40	28	
				98/60	138/50	41	21	
				115/62	178/76	55	7	
2.07	1.51	1.18	132			29		
1.38	1.78	0.01	0	160/96	192/106	20	107	Not using insulin. Sugar free
2.29		1.38	152	98/68	112/65	14	3	Insulin on night before test. sugar free
2.03		1.25	160	118/82	128/58	8	44	Insulin on night before test. sugar ++
1.64		0.81	98	120/75	148/55	23	38	Insulin on night before test. free
1.81		0.22	13	114/74	140/66	23	53	Insulin on night before test. sugar ++
2.93		1.90	185	108/60	168/80	56	28*	Insulin on night before test. sugar +++
2.01		0.85	101			24		

Recovery in 5 minutes.

## RESULTS

In the first section of table 1 it may be seen that in response to the subcutaneous injection of epinephrine into normal individuals, the blood sugar increases about 80 per cent above the fasting level, the lactic acid rises about 200 per cent and an increase of about 25 per cent occurs in the systolic blood pressure. The values for sugar and lactic acid reach a peak at the end of one hour, whereas the blood pressure begins to fall before this time. This chronological relationship for maximal effects is approximately the same in all of the groups of patients studied. While there is a rather striking uniformity in the degree of blood sugar increase among the members of the normal group, the same can not be said of the blood lactic acid although the rise of the latter is in all cases greater than 130 per cent.

In the second section of table 1 are shown the results obtained in a miscellaneous group of disease conditions. The results are qualitatively like those found in normal individuals, but it may be observed that the average increase in blood sugar and lactic acid is about half as great as in the normals. The average blood pressure response is approximately the same as in the healthy subjects. The variations in this miscellaneous group are naturally great.

The blood sugar and lactic acid curves of the diabetic patients, seen in the last section of table 1, show a still greater deviation from the normal. The average response of the blood sugar in these cases is only about 25 per cent as great as in the normal group, although the fasting level is naturally high, and lactic acid increases, on the average, only half as much as in healthy individuals. The effect on blood pressure is approximately normal. All of these patients were in a state of good nutrition.

The reactions of the blood sugar, blood lactic acid and blood pressure to the injection of epinephrine in patients with jaundice are summarized in table 2. In the first group, which embraces the cases of intrahepatic jaundice, it will be observed that the blood sugar response is about one third of the normal, while the average increases in lactic acid concentration and in systolic blood pressure are approximately the same as those found in healthy subjects. The individual variations in this group are great, and many of the patients showed a definite elevation of the initial lactic acid values as has been observed by

Adler and Lange (11), Beckmann (12), Schumacher (10) and others The cases of obstructive jaundice (the second group in table 2) due to carcinoma without liver metastases showed still less response on the part of the blood sugar and lactic acid to adrenalin, and in this group the elevation of the systolic blood pressure is distinctly less than in any other group. The high initial blood sugar values result from the presence of mild diabetes in some of the patients

The studies of patients with cirrhosis are unfortunately incomplete, but in this group the blood sugar response is of the same order as in all the other types of liver disease and jaundice investigated

In the last section of table 2 are presented the results obtained in the study of three cases of cholecystitis and cholelithiasis. The group is small but it may nevertheless be observed that in these cases too, there is an abnormally small increase in blood sugar and lactic acid after epinephrine as in the cases of obstructive jaundice, while the blood pressure rise is approximately normal

In none of the groups of cases studied was there definite molecular correlation between changes in blood sugar and blood lactic acid resulting from the administration of adrenalin. Furthermore, no constant relationship was established between the concentration of bilirubin in the serum and the response of the carbohydrate metabolism to epinephrine

#### DISCUSSION

Brill and Fitz Hugh (16) first showed that the blood sugar curves resulting from epinephrine injection are lower in the presence of liver disease than those obtained in healthy individuals, but believed that this test is of little clinical value. Kugelmann (19) in 1929 confirmed the findings of Brill and Fitz-Hugh and concluded that the test is of importance in differentiating parenchymatous liver damage from obstructive jaundice, as three cases of carcinoma of the pancreas showed a normal blood sugar response. This latter observation is at variance with our results which showed that the slope of the sugar curve in obstructive jaundice is just as low as in the cases of intrahepatic jaundice. There was no definite correlation between the severity of the liver damage and lack of blood sugar response to adrenalin. It is significant that not only in hepatic disease but also in a group of

				mgm per cent	cc	milli moles per liter				
162207	M	15	Catarrhal jaundice	+	0 7	5 44	7 17	6 94		1 7
241391	M	27	Catarrhal jaundice	6 7	1 0	5 44	6 00	6 67	6 67	1 2
236877	M	42	Catarrhal jaundice	7 1	1 0	5 72	6 72	8 00	6 72	2 2
230476	M	39	Catarrhal jaundice*	12 1	1 0	5 72	6 22	7 11	6 50	1 3
247298	M	50	Catarrhal jaundice*	25 0	1 0	6 17	6 00	6 00	5 83	-0 3
249288	M	26	Catarrhal jaundice*	18 8	1 0	5 10	5 61	6 33	6 00	1 2
81288	M	45	Catarrhal jaundice*	10 7	1 0	6 11	5 89	6 72	6 72	0 6
80130	F	34	Arsphenamine jaundice	4 1	1 0	4 89	5 50	5 96		1 0
187769	F	40	Arsphenamine jaundice	6 8	0 7	8 44	9 78	13 2		4 7
189511	M	64	Arsphenamine jaundice	8 3	1 0	4 61	4 83	6 17		1 5
228219	M	33	Arsphenamine jaundice	10 7	1 0	4 55	4 95	6 17		1 6
217054	F	32	Arsphenamine jaundice	15 0	1 0	4 39	5 50	6 89	5 27	2 5
82140	F	54	Acute yellow atrophy	20 5	1 0	6 67	6 45	6 45	6 67	-0 2
76301	F	36	Acute yellow atrophy	5 4	1 0	6 28	6 56	7 45	6 67	1 1
<b>Average</b>						<b>5 68</b>	<b>6 23</b>	<b>7 15</b>	<b>6 34</b>	<b>1 4</b>
226203	M	65	Cancer of pancreas	4 7	1 0	5 56	6 29	8 84	8 70	3 2
230777	F	52	Cancer of bile ducts	3 2	1 0	9 45	10 0	11 1	10 6	1 6
244699	M	59	Cancer of pancreas	10 9	1 0	13 1	14 8	15 2		2 1
80477	M	54	Cancer of pancreas	7 6	1 0	9 22	8 56	8 12	8 90	-1 1
82030	M	41	Cancer of pancreas	12 5	0 7	7 39	8 34	7 11		0 9
264809	M	42	Cancer of pancreas	21 0	0 8	5 44	5 89	7 05	5 94	1 6
<b>Average</b>						<b>8 36</b>	<b>8 98</b>	<b>9 57</b>	<b>8 54</b>	<b>1 4</b>
245483	F	44	Hypertrophic cirrhosis	+	1 0	5 06	7 95	9 73		4 6
81487	M	51	Atrophic cirrhosis	+	1 0	6 06	7 06	6 95	6 06	1 0
81855	M	45	Cirrhosis (?) syphilitic	6 8	1 0	6 50	6 56	7 17	6 67	0 6
69567	M	62	Atrophic cirrhosis	12 5	1 0	6 12	6 72	6 72	7 06	0 9
82774	M	62	Atrophic cirrhosis		1 0	6 84	7 44	8 67	8 95	2 1
<b>Average</b>						<b>6 12</b>	<b>7 15</b>	<b>6 85</b>	<b>7 19</b>	<b>1 8</b>
239167	M	71	Cholecystitis and cholangitis	5 0	1 0	6 61	7 50	8 73	7 78	2 1
243940	F	23	Cholecystitis and cholelithiasis	+	1 0	6 06	7 40	7 06		1 3
244726	F	58	Cholecystitis and cholelithiasis	+	0 8	5 28	6 56	6 95		1 6
<b>Average</b>						<b>5 98</b>	<b>7 15</b>	<b>7 58</b>		<b>1 71</b>

After epinephrine				100 ml. urine acid increase		Initial	Maxi mum	Systolic increase	Time after epineph rine	Remarks	
r	1 hour	2 hours		m Eq per liter	m Eq per liter	m Eq per liter	per cent	mm Hg	mm Hg	percent	minutes
2	2.74		1.87	214	99/50	133/46	33	17			
3	3.77	2.33	2.24	147	97/62	134/54	38	15			
2	2.31	1.47	1.45	168	94/64	110/64	17	50		Lactic acid excreted in urine = 17 mgm.	
3	3.04	2.19	1.82	149	105/62	140/80	33	10			
3	4.17	3.38	2.20	112	98/60	116/50	18	32			
3	1.97	1.52	1.13	135	114/70	156/60	37	34		Asphenamine 13 months before admission	
					98/60	116/50	18	42			
1	3.00		0.96	33	136/80	220/120	62	5		Operated upon 4 days later and died	
2	3.21		1.72	115	90/60	110/60	22	5		Infusion of 10 per cent glucose night before test	
3	2.11		0.89	73	130/70	136/50	5	24			
3	2.33		1.56	202	105/65	120/75	14	10			
1	5.59	4.26	4.33	343	111/70	142/74	28	11		A tourniquet was used in obtaining blood	
					105/70	115/75	10A	13		Comatose and died next day Autopsy	
										Operated upon Test done in convalescence	
1	3.11	2.53	1.83	154				26			
2	1.81	2.27	1.27	127	160/50	160/50	B			Lactic acid excreted in urine = 12.7 mgm.	
1	2.72	1.39	1.83	165	108/70	130/70	20	20		Operated upon	
5	2.19		1.13	107	102/66	112/64	10	17		Operated upon	
1	1.11	1.70	0.50	42	112/75	126/70	13	2		Died 5 days later Autopsy	
5	1.98	1.55	1.00	102	96/68	100/58	4	19		Died. Autopsy	
					132/70	140/58	6	51		Lactic acid excreted in urine = 8.5 mgm Operated upon	
1	1.96	1.73	1.15	109				11			
3	3.57		2.39	203	112/70	170/84	52	10			
					105/75	110/75	5A	18			
					128/82	130/78	2A	2			
					115/64	140/60	22	77			
					122/50	150/58	23	60		Operation	
5	2.68	2.00	1.40	109	97/56	102/66	5	24		Operation	
1	1.72		0.92	115	122/68	160/70	31	40		Proven by x ray examination	
5	2.24		0.98	78	155/60	184/50	19	30			
1	2.21		1.10	101				18			

miscellaneous diseases in which there was no evidence of liver disorder, the blood sugar response to epinephrine is subnormal.

In five of the six cases of diabetes studied, the blood sugar response to adrenalin was smaller than in any of the groups of liver disease investigated. It is possible that this is the result of the antagonistic action of insulin injected 15 hours before the experiment (9), but this time interval seems entirely too long to serve as an explanation, and furthermore, the same observation was made in one patient who did not receive insulin. In those cases in which sugar was present in the urine at the time of test, any excess sugar liberated by glycogenolysis may have been excreted in the urine without influencing the blood sugar level. The reaction of diabetic patients to adrenalin injection should be further studied.

Adler and Lange (11), Schumacher (10) and Beckmann (12) (18) as well as Wakefield and Greene (20) have demonstrated the increase in the fasting lactic acid content of the blood of patients with liver disease. Our experiments confirm this finding, but this abnormality of the blood is also found in some of our miscellaneous cases and is known to exist in cardiac disease, secondary anemias and in other diseases (14). Furthermore we observed no consistent parallelism between the severity of the liver disease and the height of the initial lactic acid level. On the basis of the theoretical considerations discussed in the introduction, it might be anticipated that following the injection of adrenalin, there would be an abnormally great accumulation of lactic acid in the blood in patients with liver disease. The results showed, contrary to expectation, that there is an abnormally small increase, both actual and relative, in obstructive jaundice and also in disease conditions not associated with pathological changes in the liver. In diseases in which there is degeneration of liver cells (intrahepatic jaundice) the findings approach the conditions which might be theoretically anticipated, i.e., abnormally high blood lactic acid levels after epinephrine, but even here the concentration does not exceed that found in normal individuals. This tendency toward lactic acid retention is, however, indicative of a failure of the liver cells to synthesize glycogen.

The work presented shows clearly that in many pathological states, including diseases of the liver, the response of the carbohydrate metabolism to the injection of epinephrine is quantitatively less than in

normal individuals, i.e., the diseased subjects act as though they had received a smaller dose of the hormone. It thus seems possible that in certain diseases, some of the effects of adrenalin are inhibited by factors still unknown. It is improbable that this is the result of poor absorption from the subcutaneous tissues as the site of injection was always thoroughly massaged and because of the long duration of the experiments. If this point of view is accepted as a working hypothesis, it renders unnecessary the assumption that the low blood sugar curves, observed in liver disease, are dependent upon depleted hepatic glycogen stores. Moreover this working hypothesis will explain the fact that the lactic acid curves in liver disease tend to be below normal instead of being elevated.

While evidence suggestive of the inhibition of the epinephrine effect on carbohydrate metabolism was found in all of the disease groups studied, the pressor effect was normal except in the cases of obstructive jaundice due to carcinoma of the head of the pancreas. In these patients the blood pressure response was definitely below normal. It is interesting to note in this group, that epinephrine also appeared to have less effect upon the carbohydrate metabolism than in any other of the disease conditions.

#### CONCLUSIONS

1 The effect of epinephrine upon the blood sugar, blood lactic acid and blood pressure has been studied in liver disease, as well as in certain other pathological conditions.

2 The rise in blood sugar is less marked in cases of liver disease, diabetes and many other pathological states than in normal subjects.

3 The blood sugar curve after epinephrine injection is in no way characteristic of liver disease and does not appear to correspond closely to the type or degree of damage present.

4 The lactic acid content of the blood in the postabsorptive period is occasionally increased in hepatic disease. This abnormality is not limited to lesions of the liver, nor is it consistently related to the severity of the process or to the concentration of bilirubin in the blood.

5 Contrary to theoretical expectations, the lactic acid curves resulting from the administration of adrenalin are frequently lower in liver disease, diabetes and certain other pathological conditions than in normal individuals.

6 It is suggested that the abnormally small response of the blood sugar and lactic acid in liver disease and certain other pathological conditions, notably diabetes, results from an inhibition of the action of epinephrine on carbohydrate metabolism

7 The pressor effect of epinephrine tends to be the same in normal individuals and patients suffering from liver disorders, except in cases of carcinoma of the pancreas with obstruction of the bile ducts where this effect is decreased

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## THE SPREAD OF RHEUMATIC FEVER THROUGH FAMILIES

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A number of epidemiological studies on rheumatic fever have been made, but in this disease, this field of endeavor is in its infancy as compared with other lines of approach. It has seemed to us, however, that here is another valuable weapon which may be brought to bear, not only because we would like to know more about the epidemiology of rheumatic fever, but it may tell us more about the nature of this obscure disease.

Knowledge concerning the actual distribution and spread of human disease, that is, its epidemiology is obtained in two ways by direct observation, and by statistical methods, the latter being wholly dependent upon the former. There has been no dearth of direct observations of one sort or another in rheumatic fever, but in spite of them, actual facts which lend themselves to statistical analyses along epidemiological lines are few. The difficulties seem to lie primarily in the nonspecific character of many of the clinical aspects of this disease. Some of its forms seem to be recognized with reasonable accuracy, but unfortunately no specific test has yet been devised to tell us when an individual actually has rheumatic fever, and we are thus unable to determine its true incidence or distribution.

With these limitations in mind we have made an attempt to study the spread of rheumatic fever through families, as an *epidemiological* problem. We have chosen the family as a unit for study, (a) because it represents a small group in which an *intimate* investigation may be carried out, and (b), because one of the striking things about rheumatic fever is its high familial incidence, of which there is ample evidence (1) (2) (3). Cohn (4) has assembled figures obtained by different observers on this point and calls attention to the fact that in so called rheumatic families, 8-10 per cent of exposed persons are

infected, as against 29 per cent in the families of healthy controls. Perhaps the most cogent observation with regard to the familial incidence of rheumatic fever is that of St Lawrence (2), who found a close resemblance to the familial incidence of tuberculosis. We do not know, however, the relative importance of certain factors which may be responsible for this high familial incidence, such as (a), an hereditary predisposition to the disease, (b), common living conditions which favor its development, or (c), direct contagion as a result of intimate contact. Such problems are in themselves so complex that our study of them can only be regarded as a preliminary form of attack in which we have begun with a single aim namely to observe the relationship as regards time of onset, which multiple cases within a given family bear to one another.

#### METHODS

*The family approach.* The idea of considering the family as a unit through which disease may spread is a concept which has proved of increasing value in the study of human disease. In the family one finds a concentration of common hereditary and environmental conditions in a group of individuals who are not only living in intimate contact with one another, but who are generally quite conscious of their group life. In the study of tuberculosis this approach has proved to be one of great value as emphasized by the work of Opie and his collaborators (5), and it is to this work that we are particularly indebted for our methods and concept of the subject.

*Selection of families.* Patients were largely selected from the Children's Cardiac Clinic of the New Haven Dispensary and from the Cardiac Division of the Children's Community Center of New Haven.<sup>1</sup>

In many instances individual children, generally accompanied by one of their parents, had been attending the Dispensary Clinic over a period of years and were consequently familiar with Hospital supervisory methods, making them a rather favorable group for the initiation of the study. Children rather than adults were primarily chosen for this work, because rheumatic fever generally makes its first appearance during childhood, and because a family of children has proved to be an easier group to handle for our purpose, than a family composed largely of adolescents and adults. These, however, were factors of choice, rather than of necessity and were not really of vital importance to our study.

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<sup>1</sup> The clinic, which has been in operation since 1924, lists about 200 patients on its roll, 90 per cent of whom are suffering from rheumatic heart disease. The Cardiac Division of the Children's Community Center (established in 1926) is now under the active supervision of one of us (R. S.). About half of its patients have been drawn from the Heart Clinic of the New Haven Dispensary.

A series of families were selected during the fall of 1928 in whom it was known that two or more members had at some time suffered from at least one of the manifestations of rheumatic fever. Selection was made on this basis because one of the main objects of the study was to observe the relationship which existed between attacks in two members of a family group. The material was further restricted to those families in which all the members had previously been under medical supervision, through the medium of the New Haven Hospital and Dispensary, for a reasonable period of years. Some of these records covered a period of twenty years. Particularly valuable in this respect were those families which had utilized the Dispensary as their "family doctor," so to speak, and had attended regularly for all trivial illnesses, as well as those of a more serious nature. As this Dispensary is the only general Dispensary at present in existence in New Haven, we were fortunate in having uniform and centralized records on this group of patients. Occasionally it was necessary to draw upon the records of other Hospitals<sup>2</sup> in order to fill in certain gaps.

*Method of family study.* When all available records of past illnesses in one of the selected families had been secured, an appointment was made through the agency of a social service worker for a visit to the home of the family, with an explanation of the purpose of the visit and with special emphasis on the fact that the entire family group should be at home at the appointed hour. If any member of the family happened to be under the care of a practicing physician, the physician was either seen personally, or called on the telephone by one of us, in order to explain our reasons for questioning or examining his patient. Subsequently a copy of our findings on this and other members of the family was sent to the physician, if he so desired. A visit was then made by one of us to the home. The living quarters were inspected and investigated. Each member of the family who had not been seen in the Dispensary within a few weeks' time was submitted to a physical examination and adults were questioned with a view to the filling in of gaps in our records of their personal, social and past histories. Careful and exacting inquiries were made with regard to changes in the family environment, such as the sites of previous living quarters, and the dates on which the family had moved. Other members of the household who had become established as members of the family group, whether relatives or otherwise, were included in the studies. Subsequent to the initial visit the home was visited by one of us at intervals of two months, or more frequently if illness appeared in any member of the family group.

It will be seen that the attempt has been made to carry on the study in an intimate and personal manner, as we were anxious to establish close contact with the conditions under which the disease naturally develops, rather than to study patients who were isolated from their environment. Social workers<sup>3</sup> have been

<sup>2</sup> We are particularly indebted to Mr C H Dabbs, Superintendent of Grace Hospital, New Haven, for the privilege of examining some of the medical records of that institution.

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utilized for the arrangement of appointments and for the gathering of non-medical data, but all visits made for purposes of ascertaining data on the *health* of the family, were made by one of us. This mass of detailed work has necessarily required the restriction of the families under supervision to a relatively small number, which to date numbers fifteen.

*Charting results* In order to give a graphic representation of the life history of these families and of the incidents with which we have been concerned, a diagrammatic chart was designed for each family. The method of charting is shown in figure 1. This figure requires a certain amount of explanation not shown on the diagram. On the right of the figure, the age of the family group, starting at the bottom with the birth of the parents, has been marked in decennial, biennial and annual periods, extending up to the point in time to which the study has been carried. The life span of each individual in the family is designated by the vertical columns inclosing space for the chronological tabulation of illnesses. Changes in family environment, as noted by change in address, are designated by horizontal lines, the removal of one individual from the family environment by enclosing the space about the life column.

On the left are the legends for tabulating illnesses. These are recorded as follows: the right side of the column is for the entry of the legends representing symptoms, signs, etc., which *may or may not* be related to rheumatic fever, the left side is for definite evidences of *activity* of rheumatic fever, shown as black blocks, varying in size according to our estimation of the degree and duration of the period of activity. The center of the life space is for the entry of rheumatic cardiac lesions, which we have come to recognize as one of the most important evidences of rheumatic fever now at our disposal. The first appearance of carditis has been designated in the form of a small Greek cross, and continued evidences of such a lesion, by a vertical line projecting from its top. The number of times which each individual has been seen by a physician at home, in the Dispensary, or in a Hospital, is shown by the black dots and rectangular figures aligned on the left of the life column. It is to be emphasized that these legends, marking the times which the patient has been under medical observation, do not always mean that a complete or even an adequate physical examination was performed at each visit. As a whole the dots represent the type of examination which is apt to be performed upon patients attending the various clinics of a Dispensary. Visits to the eye clinic for purposes of refraction, and to the dental clinic for the extraction of teeth, have not been included.

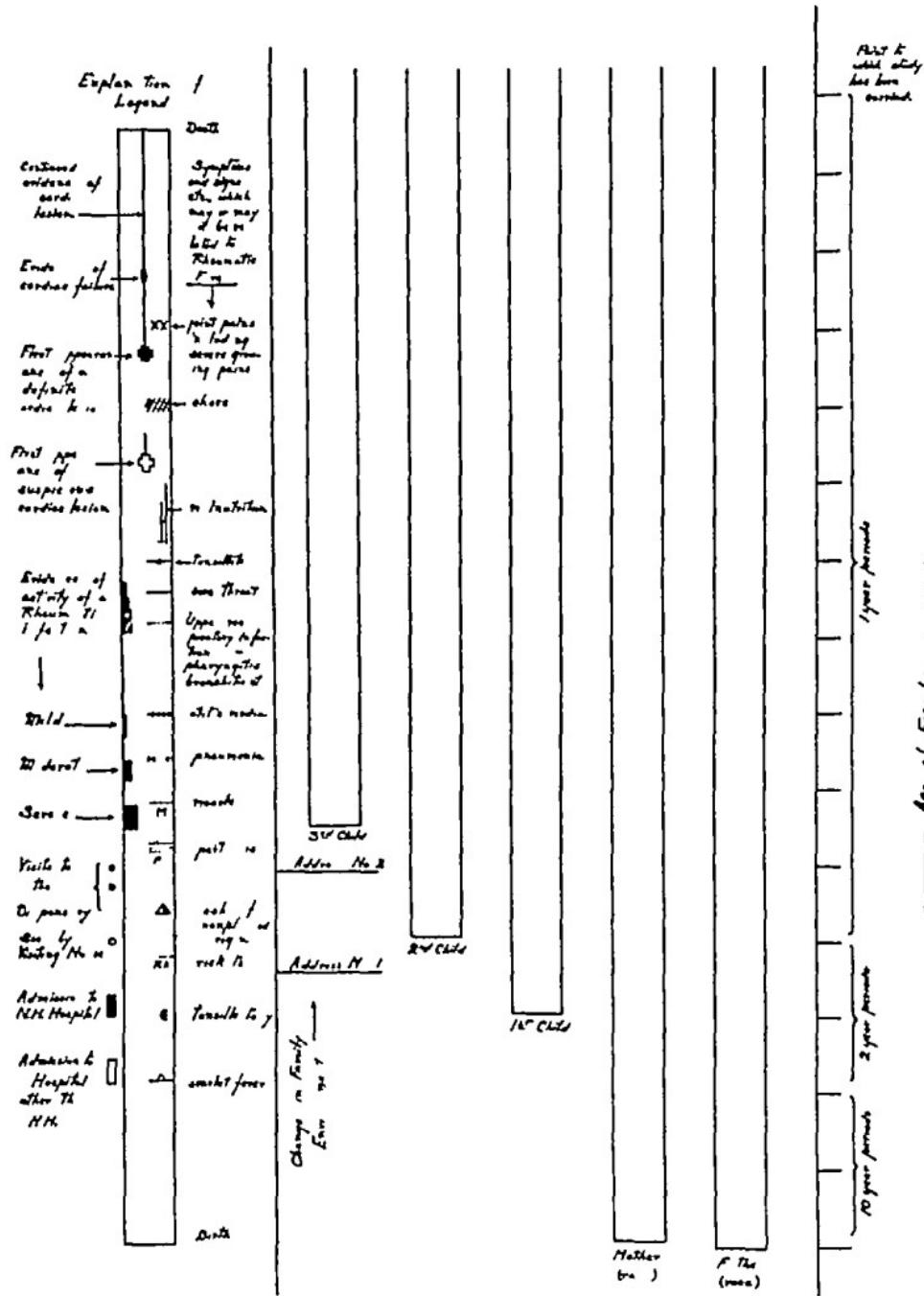


FIG 1 EXPLANATION OF CHART FOR RECORDING FAMILY DATA

## RESULTS

To date fifteen families have been subjected to the type of study outlined above, of which five examples are shown. In some, data have been reasonably complete, in others there are large gaps. A glance at the charts showing the number of times different members of the family have been under medical observation will give some idea of the degree of completeness with which the data have been gathered.

A repetition of the story of the illnesses experienced by each family will not be attempted for it can be ascertained from the chart. However, a few explanatory statements will be made to supplement the data on each chart.

In figure 2 (family 2—Mn) are recorded the data from a family with four children. Unfortunately intimate knowledge of the history of both parents is lacking. The father, gave a clear cut history of having had what he was told was rheumatic fever when 10 years of age. At that time he was confined to bed with fever and joint pains for a period of months and was told that his heart was affected. During the subsequent twenty years he experienced no untoward symptoms and in recent years had been able to pursue the occupation of truck driver until October 1928, when he began to suffer from a sore throat lasting over a period of two months, accompanied by bouts of fever. When first examined in January 1929, he was still suffering from his throat infection and also showed an over-active heart and evidences of mitral stenosis. Subsequently he improved and has been without symptoms for more than a year. Coincident, or a few weeks previous, to the father's illness the oldest child began to suffer from joint pains, loss of weight, bronchitis, and on two examinations a systolic murmur was detectable, which was not present in subsequent examinations. In December 1929, also coincident with his father's and sister's illness, the younger brother (William) developed outspoken evidences of rheumatic infection with carditis which persisted for a year. He was removed from the family environment and has been subsequently kept at the Children's Community Center.

We have, therefore, three more or less synchronous attacks of illness in this family, two of which represent probable periods of activity of rheumatic fever and one of which (William) represents a definite period of rheumatic activity. In the father the illness was a recurrence, in the two children the illness probably represented the first manifestations of rheumatic fever.

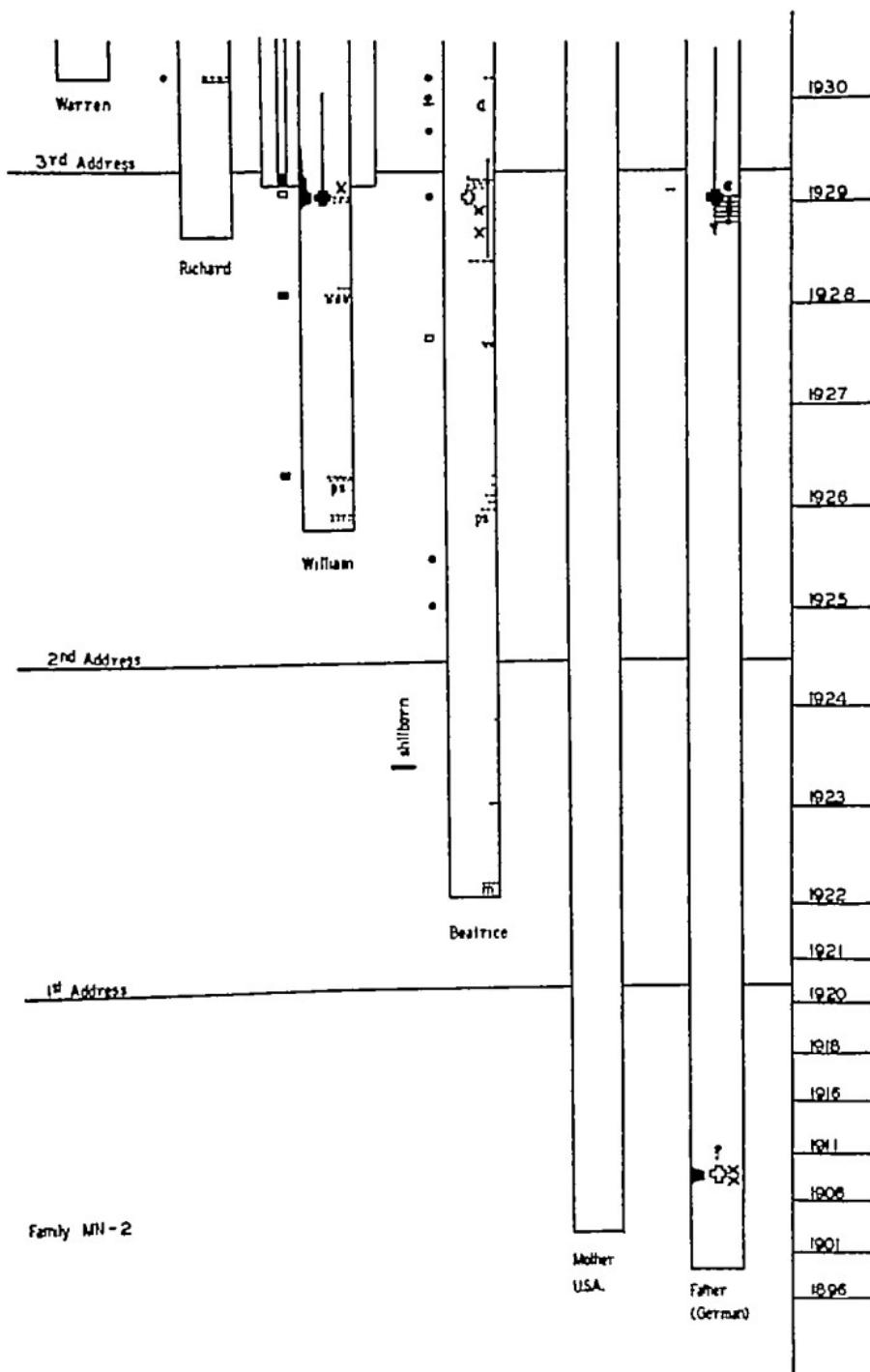


FIG 2 FAMILY 2

Figure 3 (family 4—Ds) Both of the parents were Jewish and had apparently lived at the same address since the birth of the first child They were poverty stricken and lived practically in one room during this entire time In 1925, Jacob, the fourth child, developed rheumatic fever and was admitted twice to the New Haven Hospital Evidences of severe carditis did not appear until six months after his initial attack, but were severe enough to require his removal to a Cardiac Home Interestingly enough at about this time a mitral lesion was picked up in the mother, although repeated previous examinations during her visits to the Dispensary for prenatal and other types of care, had failed to reveal this finding No other members of the family showed evidences of rheumatic fever during the two years subsequent to its first appearance However, in the fall of 1928 the mother entered the hospital with a flare-up of her infection, suffering from severe carditis and cardiac failure At the time of her admission she had been suckling Abie, the eighth child—aged one year He was also brought to the hospital with the mother and found to be suffering from fever and a skin rash, originally thought to be measles His illness was brief and no satisfactory diagnosis was reached During the next year and a half, he was again admitted to the hospital, his next older brother, who slept at home in the same bed with him, was also admitted to the hospital three times during this period, suffering from bronchitis and skin rashes of unexplained origin Transient evidences of a suspicious cardiac lesion were picked up in both of these children in the course of the hospital studies

We have in this family, observations on (*a*) an attack of rheumatic fever in one child (Jacob), associated with the appearance of signs of rheumatic carditis in the mother, and later (*b*) the development of illness in two infants, one of which occurred synchronously with an attack of recurrent rheumatic fever in the mother Whether the symptoms in these infants actually were those of rheumatic fever is questionable, but the balance of evidence would indicate that such was the case

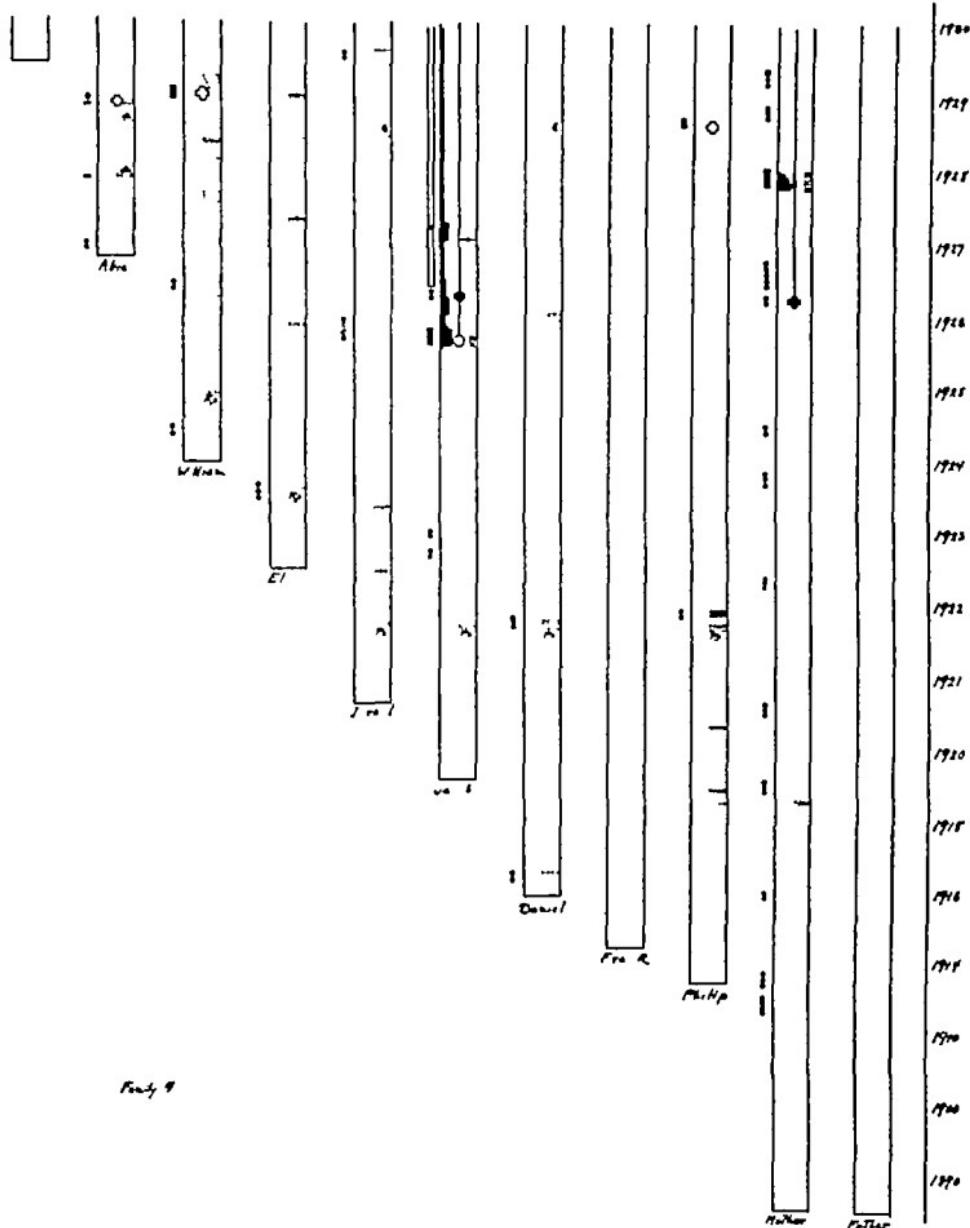


FIG. 3 FAMILY 4

Figure 4 (family 12—Me) Both father and mother were French Canadians and in 1917, seven years after their marriage, had moved to New Haven from Chicopee, Mass. In 1921 the father, who was in the insurance business, died suddenly from what was thought to be Angina Pectoris. The family was left in rather straightened circumstances and became Dispensary patients about this time. As medical assistance had been previously rendered by private physicians we have no first hand records of the children's previous illnesses or examinations. In 1922, the three oldest children suddenly began to suffer from active manifestations of rheumatic fever, all within a period of six weeks. The fourth and youngest child also showed mild evidences of ill health, manifest by joint pains and loss of weight. The subsequent history of this family is relatively clearly shown upon the chart. The oldest boy enlisted in the United States Navy but was discharged unfit, after spending a large part of his time in the Hospital. The youngest girl has shown persistent evidences of malnutrition to date with occasional transient joint pains. It seems probable that she has been suffering from low grade rheumatic infection although the diagnostic criteria are perhaps not quite adequate.

The simultaneous onset in 1922, of what appear to be four cases in four children, is striking.

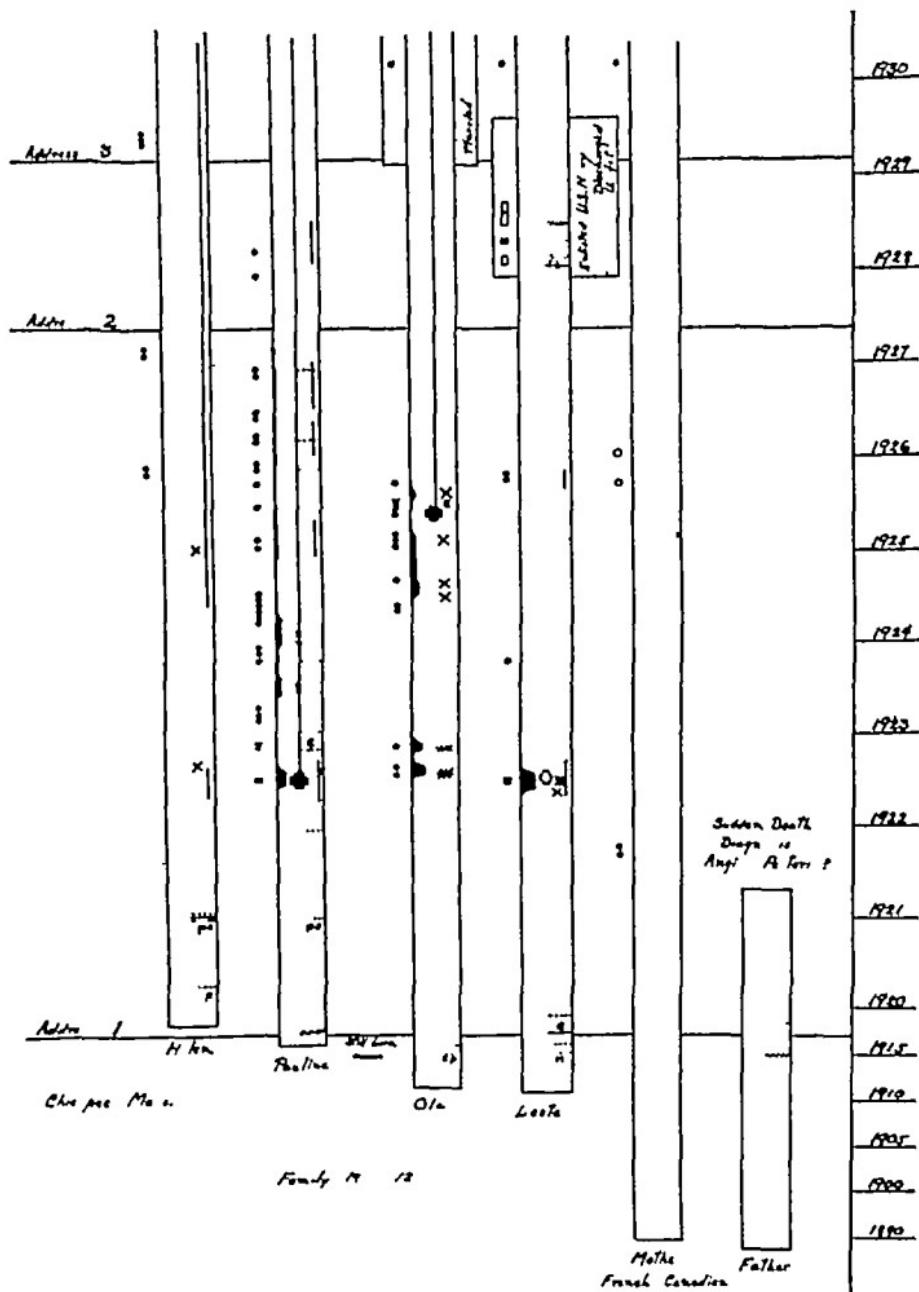


FIG. 4 FAMILY 12

Figure 5 (Family 6—De Gn) Both father and mother were Italian During their early married life they had shifted from one city address to another but had finally moved to the fifth address, a house on the outskirts of the city, because they thought their lodgings in town were unhealthy It was at the town lodging (address no 4) in 1922 that the first manifestation of rheumatic fever had appeared, in the form of a brief attack of chorea in the second child, and in the development of an early cardiac lesion in the third child The three youngest children also suffered from what seemed to be minor illnesses at this time (1922), such as tonsillitis, bronchitis, and otitis media Subsequently only one child has developed severe carditis but the three youngest children have developed suspicious cardiac lesions, one of them in an attack of recognizable rheumatic fever It is also interesting that during the period when the children were showing evidences of low grade rheumatic fever the mother was being treated at the Dispensary for infectious arthritis

It would seem, therefore, that in 1922 an epidemic of illness had swept through this family, which took the form of rheumatic fever in two children, in the others it might be logically described as upper respiratory infection Subsequently some of the children in the latter group also seem to have developed rheumatic fever

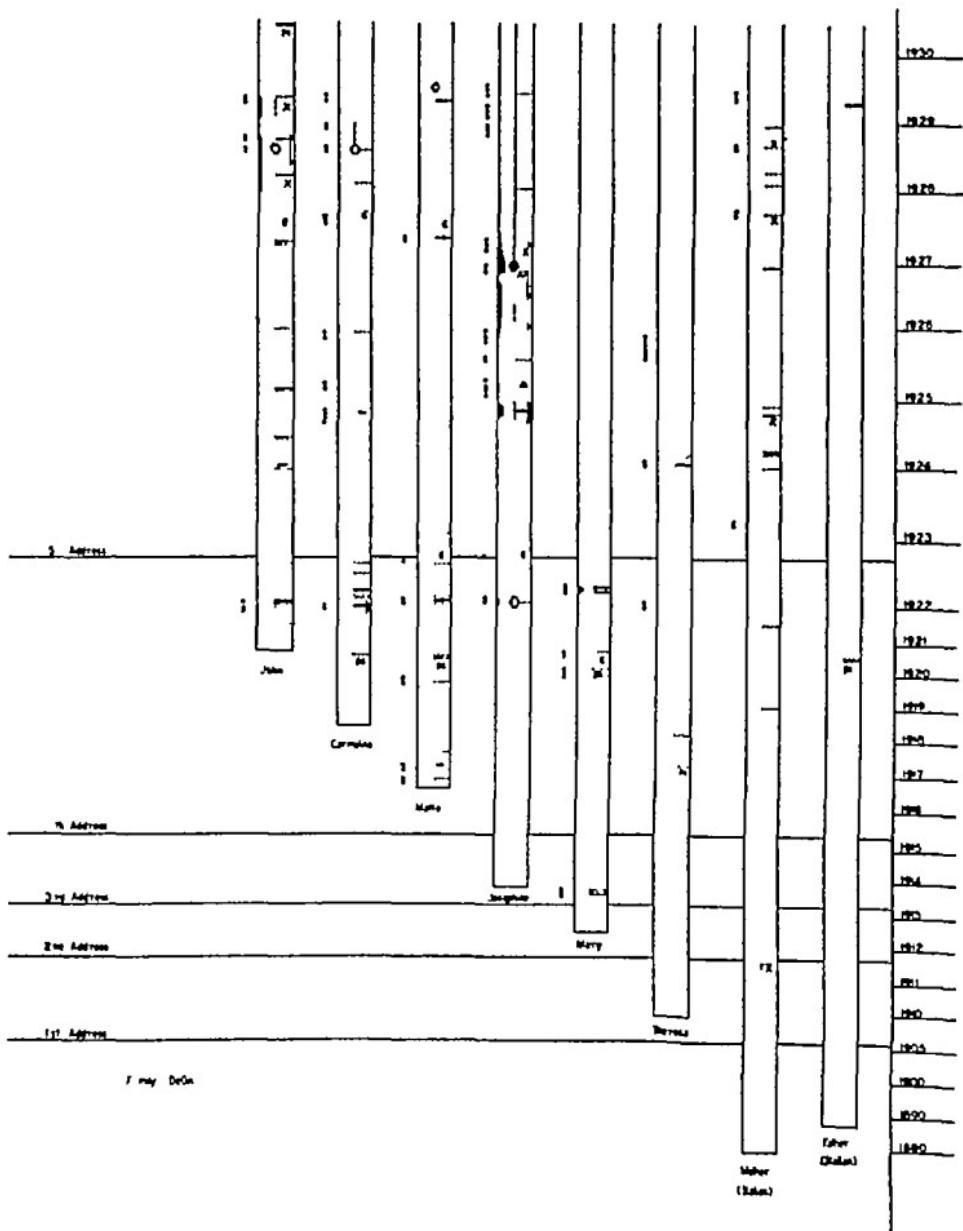


FIG 5 FAMILY 6

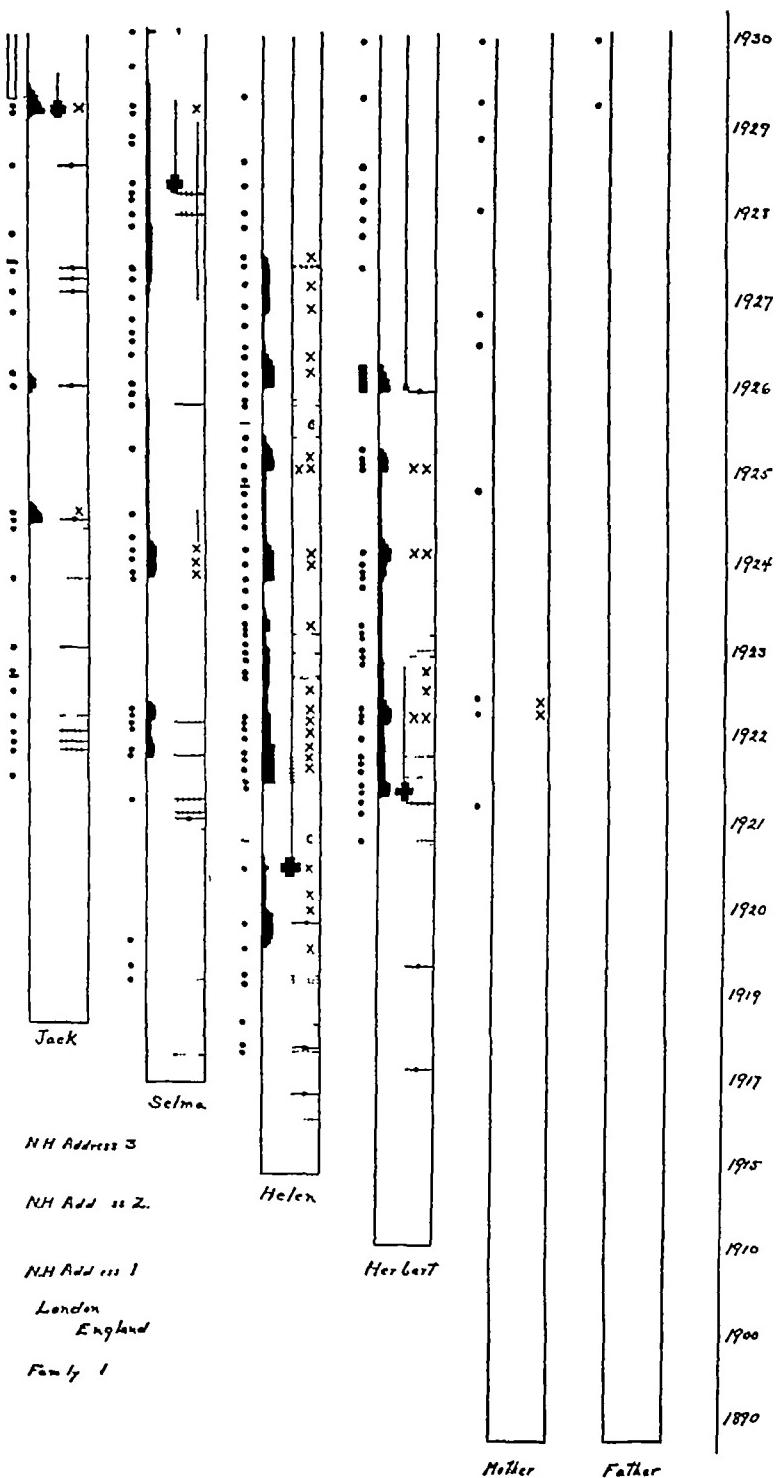


FIG. 6 FAMILY 1

Figure 6 (family 1—Bv) A Jewish family which had emigrated from London to New Haven in 1914 In neither parent were there evidences of the disease All of the four children have been under fairly close observation for at least 10 years, all had rheumatic fever, and all have cardiac lesions In three the transient nature of cardiac signs are well shown

An interesting feature in this group is that all of the children suffered from a series of rather severe, upper respiratory infections over a period of 2 to 6 years, prior to the development of recognizable evidences of rheumatic fever Further more synchronous waves of illness have swept through this family from time to time in which two or more children have shown simultaneous periods of activity of their rheumatic infection Interestingly enough, during one of these periods the mother was attending the New Haven Dispensary for an illness thought to be infectious arthritis.

#### DISCUSSION

Our observations have been made upon only fifteen families and are subject to many criticisms of which the most obvious is, that the historical data concerning certain of the individuals under observation are somewhat sketchy Furthermore the interpretation of our findings may only apply to the conditions under which the study has been made, namely in families in which the incidence of the disease is high

Our major aim was to observe the relationship as regards time of onset, which multiple cases within a given family bore to one another The results indicate that the appearance of either primary or recurrent bouts of rheumatic activity in certain members of these families has been frequently accompanied by the almost simultaneous (or the immediately subsequent) appearance of the disease in other members of the family This fact does not appear remarkable in view of the many reports of epidemics of rheumatic fever which have been recorded, in houses (6) (7) (8), in barracks (9) (10) (19), in schools (11) (12), and communities (13)

Another fact which has been observed is, that not only does the disease frequently make its first appearance almost simultaneously in several members of a given family, but periods of recurrent activity seem to sweep through a family in synchronous waves (cf fig 6) This latter observation is also in confirmation of a now fairly well recognized occurrence in juvenile rheumatic populations, namely, the epidemic manner in which severe *flare-ups* of rheumatic activity may

occur Boas and Schwartz (14) have described two such "epidemics" occurring in a ward of convalescent rheumatic children Hiller and Graef (15) have also described a similar situation in a camp for children with rheumatic cardiac disease These examples do not represent true epidemics of rheumatic fever in the sense that new cases suddenly appeared in a previously uninfected population Rather they are examples of simultaneous periods of "reactivation" apparently following, after a definite interval, an epidemic of some upper respiratory infection Our interest has been to differentiate such occurrences from the simultaneous appearance of new cases in a previously uninvolved population, although we have occasionally found difficulty in determining whether some of our cases actually occurred in virgin soil or not

Still another interesting finding associated with these family epidemics has been the appearance of *nonspecific* types of illness, such as (a) bronchitis, bronchopneumonia, skin rashes, and (b) rheumatoid arthritis The first group of conditions have been prone to appear in children who have not previously had rheumatic fever, often acting as a "precursor," so to speak of recognizable forms of the disease Such phenomena might be explained on the basis of differing age and immunological expressions of the *host* to the *rheumatic virus*, for there are few diseases in which the age of the patient determines the clinical manifestations of the disease more than rheumatic fever, and it is to be recalled that in families such as the ones selected above we are dealing with groups of individuals of widely differing ages It is also to be recalled that the limits of the clinical picture of this disease have not as yet been determined Our epidemiological studies therefore offer an opportunity to broaden conceptions with regard to the clinical manifestations of this disease, particularly in what seem to be first attacks in very young children

An interpretation of these observations from an etiological point of view offers certain complexities which do not readily lend themselves to analysis However, previous evidence and the evidence furnished above, support the fact that rheumatic fever is an infectious disease We recall, however, that an appreciation of the pathogenesis of this and other infectious diseases embraces at least three major factors (i) the incitant, (ii) a susceptible host, (iii) paths which facilitate the

bringing together of 1 and 11. In rheumatic fever we are not sure of the nature of any of these three factors, although it would seem that the second factor—the nature of susceptibility, must be of the utmost import in this disease, and one which has led some investigators to champion rheumatic fever as a disease in which heredity plays an all important rôle (16) (17), and others to champion the rôle of environment (18). That environmental factors to which a whole family is exposed must be of some importance is shown by our observations that flare ups of *recurrent* activity of the disease seem to sweep through a family in synchronous waves. Our findings are in accord with at least three current theories which have to do with susceptibility in this disease. (A) That crowding and the presence of any one of a variety of upper respiratory infections is the important factor in spreading the unknown virus by droplet infection (19). (B) That rheumatic fever is essentially an environmental disease (18), in which unknown factors, perhaps having to do with climatic conditions (20), housing conditions (21) (22), or nutritional factors (23), pave the way to development of an infection of unknown etiology. (C) That allergic phenomena elicited by bacteria, of the streptococcus group, may figure with great importance among the factors which predispose to the disease (18) (19). All three theories, together with the view that heredity<sup>4</sup> plays an important predisposing rôle might fit in with the limited facts which we have observed, and their further elucidation must await more extensive observation.

Nevertheless whatever may be the true nature of certain hypothetical factors, which seem to be of great importance in predisposing to the disease, our results indicate that, given the conditions under which the disease seems to flourish, it acts epidemiologically like an infection, often producing explosive primary or recurrent outbursts of activity in several individuals, who may show extremely different types of host response. During these epidemic waves, and in rheu-

<sup>4</sup> An analysis of the rôle of heredity may be more satisfactorily approached when control studies of this type (which are now in progress) are directed against groups of unrelated individuals who are living in close proximity to one another. We have already found two tenement houses in New Haven in which manifestations of rheumatic fever have appeared, more or less simultaneously, in several children representing two different families.

matic families in general, children between the ages of 4 and 12 seem to be particularly prone to develop the infection. The ages at which this susceptibility reaches its height and begins to diminish, will be discussed in a subsequent paper (26).

#### SUMMARY

1 The spread of rheumatic fever through families strongly suggests that we are dealing with an infection, in which the important rôle of environment, and of an hereditary predisposition to the disease, is as yet undetermined.

2 The sudden appearance of what seem to be both primary and secondary outbursts of rheumatic activity in certain individuals has been frequently accompanied by the appearance of recognizable forms of the disease in other members of the family group, and not infrequently by the simultaneous appearance of nonspecific types of illness such as bronchitis, bronchopneumonia, skin rashes, and rheumatoid arthritis. We have therefore an unusual opportunity to broaden our conception of the disease by this approach.

3 Not only do several cases seem to be initiated simultaneously, but periods of recurrent activity of the disease seem to sweep through a family in synchronous waves, during which time new cases may also appear.

4 These findings have been discussed in the light of current theories dealing with the pathogenesis of rheumatic fever.

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## AGE SUSCEPTIBILITY TO FAMILIAL INFECTION IN RHEUMATIC FEVER

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One expression of our changing views with regard to rheumatic fever is the increasing importance being attached to juvenile forms of the disease. Older figures on the age incidence of this disease were based largely upon the incidence of both the primary, and recurrent attacks. Such figures minimized the importance of childhood as the period of greatest susceptibility. For instance in Rolly's monograph on rheumatic fever (1), published in 1920, he listed the age incidence of all periods of activity in a series of 1450 cases, and from these figures found, that only 4 per cent of the attacks occurred in the first decade of life, 40 per cent in the second, 39 per cent in the third, etc. The conclusion reached was that rheumatic fever found its highest incidence in the second and third decades of life. If, on the other hand, a series of cases is analyzed for the age incidence of *first attacks*, a very different result is obtained, showing that by far the greater number of initial manifestations of the disease occur in the first decade of life. This fact now seems well established among groups of patients attending hospital clinics in large cities, although we have no data on rural populations.

Our present views with regard to the age incidence of first attacks may now be briefly reviewed as follows. It is apparently rare, although not exceptional, to find the disease during the first year of life. Most of the cases which have been recorded in young infants were being nursed by mothers who were suffering from active phases of the disease, a fact which is of importance not only from the standpoint of age susceptibility but also from that of the transmissibility of the disease. Equally important are the isolated case reports, mentioned by Rich-dorf and Griffith (2), of apparent congenital acquisition of rheumatic

fever These describe instances of the disease occurring in women who were pregnant, to be followed by the birth of an infant who within one or a few days apparently showed manifestations of rheumatic fever

After the age of one year there is a rapid rise in the incidence of first attacks, reaching a peak between 5 and 15 years In England, Poynton's series (3) showed an average age at onset of 7, Coombs' series (4) of 10 In New York City, Wilson et al (5), who made extensive

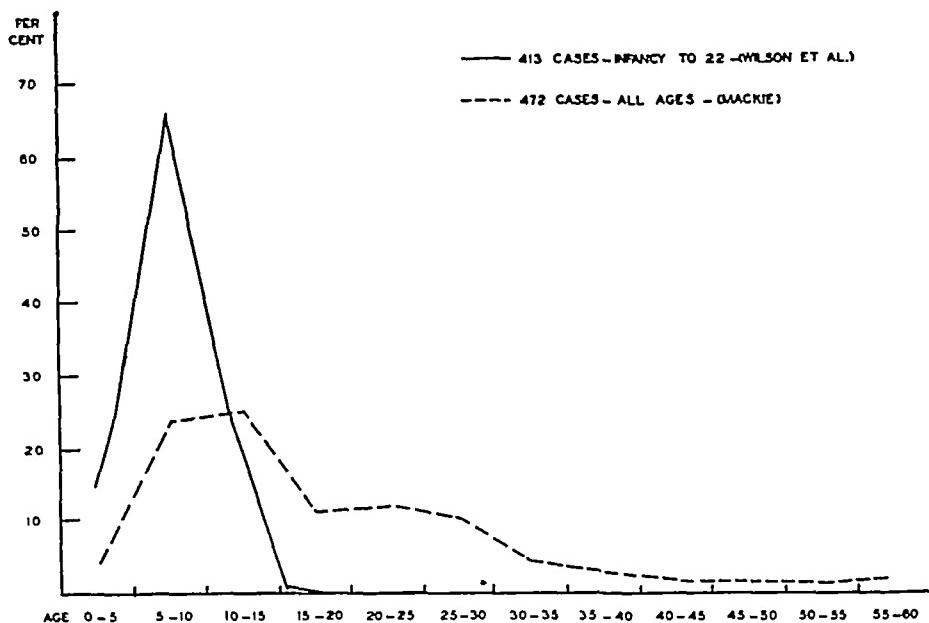


FIG 1 THE AGE INCIDENCE OF FIRST ATTACKS OF RHEUMATIC FEVER

statistical studies on a series of 413 children, ranging in age from infancy up to 22 years, observed during a ten-year period, that in 98.4 per cent of their cases, initial infection occurred before the age of 15, the average age at onset was 7.3 years Mackie (6), also in New York City, has attempted to determine the age incidence of initial attacks from a series of 472 individuals, drawing on historical data for his facts The results of both of these analyses are shown in figure 1 Their differences may be dependent both upon the manner in which the data were obtained, and the manner in which the series of cases

were selected, but both emphasize the 5-15-year period as one in which the disease is generally acquired. The attempt has also been made to decide from these and other data, just where susceptibility to rheumatic fever reaches its height and where it begins to diminish. Wilson et al (5) believe that rheumatic infection concerns itself primarily with children of the grade school age of 6 to 14, and that about or before the age of twelve, the tendency to infection begins to diminish.

Caution must be exercised in making generalizations from the figures quoted above when we recall for instance that Wilson's group was drawn from a juvenile population in which the factor of initial selection is of the utmost importance, for if rheumatic fever adheres to the principles guiding the spread of other infectious diseases, it owes its incidence to many factors, including the facility with which it may be spread, as well as the size and character of the susceptible population.

With these points in view it has seemed interesting to analyze this situation as it occurs in families in which the incidence of the disease is high. Here again one is dealing with a rigidly selected population but the data would seem to be valuable because, besides determining the age incidence of initial attacks in the family series, one has the further opportunity of listing the ages at which exposure to familial infection, first and subsequently occurred.

#### METHODS

The method of selecting the families and of assembling and recording the data, has been described in the preceding paper (7). Fourteen families were available for the present study, representing 99 individuals—24 parents and 75 children, covering an age group extending from infancy up to 50 years, although a gap occurs between the ages of 18 and 33. In order to facilitate our statistical analysis, a chart was devised on which each of the individuals has been designated by a vertical line, corresponding in length with the individual's age. These lines have been punctuated by legends, designating the following three episodes (a) *Exposure to familial infection*. This has been defined as follows: when any member of a given household suffered from an active phase of rheumatic fever it was presumed that all of the

other members living at home at that time, were exposed. As some of these active phases were prolonged, it was often difficult to say just when exposure ended and whether multiple exposures occurred. Such prolonged attacks have, however, been recorded as a single

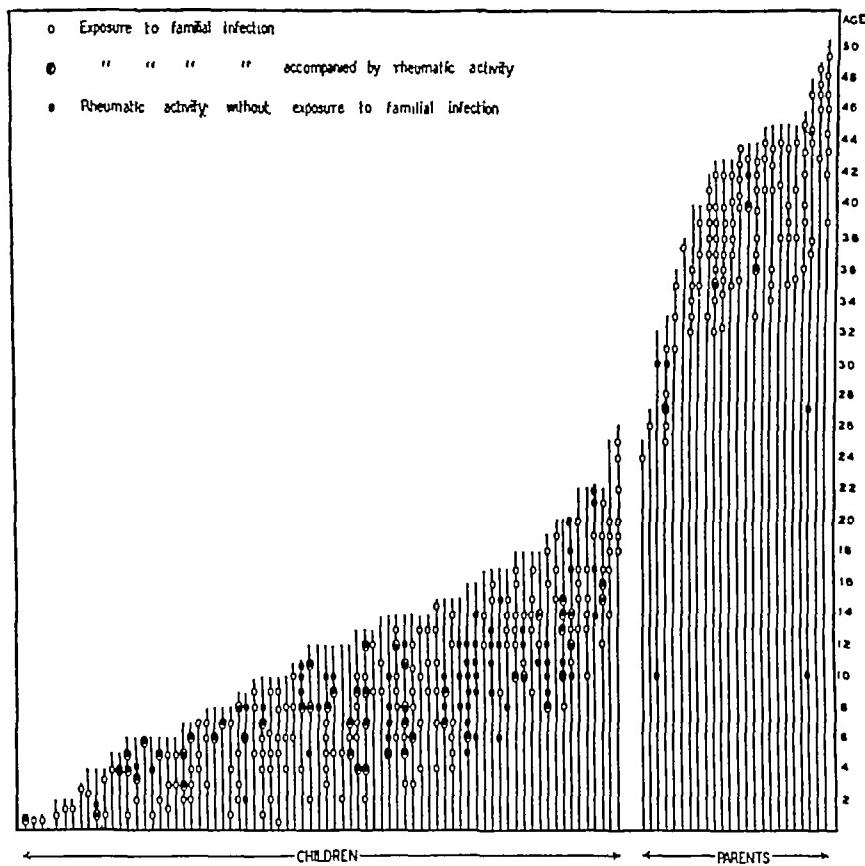


FIG 2 THE AGE DISTRIBUTION OF ATTACKS OF RHEUMATIC FEVER IN 99 INDIVIDUALS, WITH SPECIAL REFERENCE TO ACQUISITION OF THE DISEASE FOLLOWING FAMILIAL EXPOSURE

exposure dated from the onset of the attack (b) *Exposure to familial infection as defined above, with acquisition of the disease* Instances of this kind are represented by the development of an active phase of rheumatic fever in any member of the household within a period of 4 weeks following the development of the disease in another member of

the family. In a few instances several cases have appeared almost simultaneously in a given family. All of these cases may have been examples of acquisition of the disease from extra-familial sources. Under these circumstances, I have assumed as an arbitrary principle, that one of the cases represented extra-familial infection, the others, intra-familial infection. (c) *Acquisition of the disease, not explainable on the basis of familial infection.* This has been defined as the appear-

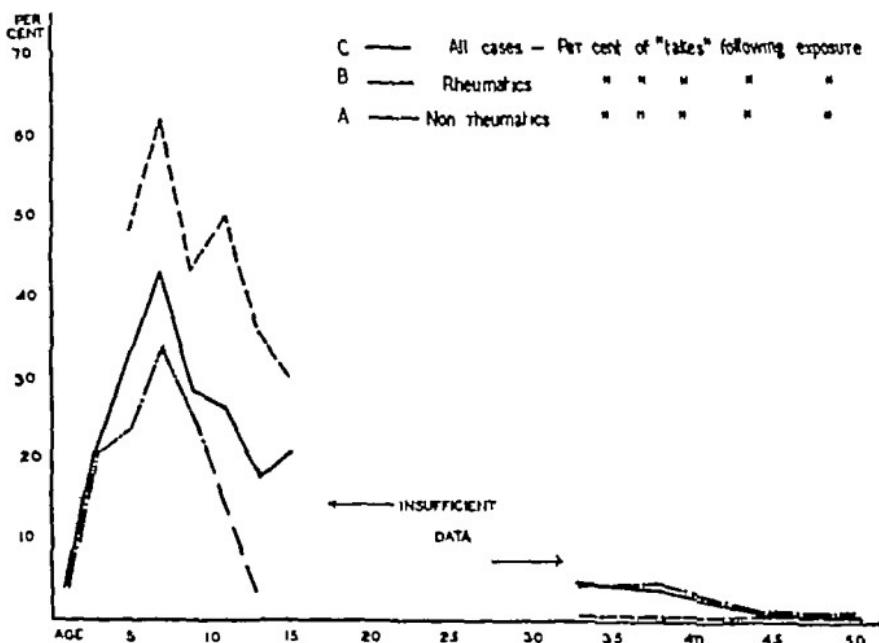


FIG. 3 THE PERCENTAGE RATES AT WHICH INDIVIDUALS OF DIFFERENT AGES ACQUIRED RHEUMATIC FEVER, FOLLOWING FAMILIAL EXPOSURE

ance of a primary or recurrent active phase of the disease in a member of a household, in which no other members of the family group had shown active evidences of rheumatic fever within a period of 6 weeks.

#### RESULTS

In figure 2 is shown a chart constructed on the basis just described. It shows the age distribution of the cases, and roughly the period during which they have been under observation. It shows a concen-

previously suffered from any manifestations of the disease, rheumatic fever was acquired in association with familial exposure, at the rate of 25-35 per cent

Among children between 5 and 12 years of age who had already survived an initial attack, recurrences were noted in association with familial exposure at the rate of 35-60 per cent, decreasing as age increased, but representing a markedly enhanced susceptibility during adolescence

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# THE PATHOLOGIC PHYSIOLOGY OF PELLAGRA

## I TABULATED CLINICAL AND PHYSIOLOGIC DATA

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(Received for publication October 27, 1930)

Pellagra is almost unique among major diseases in the scarcity of accurate information concerning it. There is still uncertainty about its etiology, the nature of its pathologic lesion is obscure, the diagnosis in atypical cases is a mere matter of opinion, and there are no adequate criteria for cure or for prognosis. The pellagrin who remains ill for a prolonged period may be suffering from chronic pellagra or may be suffering from some mysterious sequel rather than the disease itself. Prognosis is based chiefly on mass statistics largely unmodified by the clinical features of the individual patient. Even the causes of death are little understood. Pellagrins frequently die unexpectedly when apparently convalescent. The treatment at present is highly unsatisfactory. More than 25 per cent of the pellagrins admitted to the New Orleans Charity Hospital die in the hospital, in spite of treatment along the most approved lines. The treatment is dietary and symptomatic. The dietary is based on inexact knowledge of the dietary fault to be overcome. The symptomatic treatment rests on imperfect knowledge of the disturbed physiology represented by the symptoms. Pellagra takes a variety of clinical forms, so that some writers have suggested that what is called pellagra is a mixture of diseases rather than an entity. If typical skin lesions are accepted as a *sine qua non* there can be little doubt that pellagra is a unique disease for there is hardly any lesion in medicine more distinctive clinically. Even with classic skin lesions the cases fall into sharply contrasting groups. The mechanisms which bring this about are quite mysterious. The studies upon which this and subsequent papers are based were undertaken with the belief that there was no better mode of attack on many of the

TABLE I  
*Data relative to forty-two pellagrins analyzed in this and succeeding papers*  
 (For significance of code designations, see footnote to table)



TABLE 1—Continued

Case number	Race	Sex	Age	Interval	Duration	Severity	Present state	Diarrhea	Skin lesions	Remarks																																
										code	code	code	code	code	code	code	code	code	code	code	code	code	code	code	code	code	code	code	code													
64	C.	M	46	—	4	2	1	DE	4	2	1	0	0	0	A	0	2	72	49	5	34	54	260	2	740	1	470	2	87	3	07	5	94	10	7	3	4	492	71	138		
15	C.	F	60	—	4	3	3	U	4	3	1	0	0	0	A	0	0	0	51	0	28	0	4	420	3	150	1	240	2	65	3	015	66	11	5	4	5	524	62	147		
16	C.	M	61	—	1	3	3	B	1	2	1	0	0	0	A	0	0	0	37	6	31	5	3	100	2	050	980	3	31	2	716	0	2	9	8	5	4	53	41	Died 1 day later		
17	C.	M	61	—	17	5	6	H	0	0	0	0	0	0	A	0	0	0	42	2	29	8	2	610	1	830	750	2	96	2	305	26	10	1	4	6	500	41	Improved			
18	C.	M	55	—	3	2	3	UH	10	2	1	0	0	0	A	0	0	0	39	9	38	0	3	720	2	290	1	400	4	181	67	5	85	8	5	12	0	336	22	Skin lesions probably re-current		
19	W.	F	46	—	7	4	2	1	DE	4	2	0	0	0	A	0	1	61	40	3	32	3	3	390	2	290	1	080	3	34	1	96	5	30	9	8	5	1	634	61	Discharged improved	
20	C.	M	44	—	7	3	3	D	3	1	1	0	0	0	A	0	1	70	47	2	30	7	5	500	3	790	1	680	3	78	2	276	0	5	9	5	3	2	71	616	62	Syphilis
21	W.	F	29	—	12	3	2	DU	12	2	1	0	0	0	H	0	1	38	0	34	0	3	530	2	350	1	170	2	63	1	93	4	56	10	7	4	8	530	59	140	Recurrent skin lesions Active delirium 1 week later, then died	

22	C.	P.	11	-	12	2	3	D	12	2	1	0	D	0	-	0	0	43	21	228	0	-	-	2	22	3	015	27	85	52	432	53	116	Died 1 hour later			
23	C.	M.	10	-	16	3	3	D	4	2	1	0	D	0	0	0	0	35	24	130	112	570	1	770	81	2	114	92	84	53	464	51	123	Improved			
24	C.	F.	34	-	5	3	1	H	12	3	0	0	D	0	A	0	0	61	48	715	214	240	3	090	1	120	2	112	285	40	85	38	586	53	154	Stricture of rectum Discharged unimproved	
25	W.	F.	16	-	12	5	1	H	4	1	0	0	D	0	A	0	0	56	36	334	532	860	1	830	990	4	872	207	02	11	4	57	53	153	Pyrexia. Diarrhea had stopped 3 months before admission		
26	W.	F.	23	-	6	2	1	H	0	0	1	0	A	0	0	0	0	36	36	810	612	640	1	810	810	4	012	13	17	10	76	62	614	57			
27	C.	F.	39	-	4	2	2	DU	0	0	1	0	D	0	1	0	0	37	31	32	53	159	2	110	1	000	1	102	17	16	33	11	34	604	50		
28	W.	F.	26	-	5	2	1	H	0	0	1	0	A	0	0	0	0	37	37	535	512	600	1	700	880	4	142	15	16	29	11	1	48	570	60		
29	W.	M.	36	-	4	2	1	E	0	0	1	0	E	0	0	0	0	67	52	236	04	940	3	110	780	4	032	37	16	35	11	8	32	620	58		
30	W.	M.	44	-	6	3	1	E	0	0	1	2	E	0	0	0	0	57	57	735	54	550	2	890	1	610	3	91	2	37	6	28	11	8	34	510	55
31	C.	M.	53	-	10	1	1	E	0	0	0	0	E	0	0	0	0	70	59	043	45	580	3	150	2	420	3	60	3	016	61	98	34	492	53		
								D	0	0	0	0	D	0	0	0	0	58	58	141	65	680	3	220	2	420	2	90	2	85	80	91	32	600	47		

TABLE I—Concluded

Patient Data												Treatment Response																			
Initial Assessment												Follow-up																			
Demographics						Clinical Findings						Laboratory Findings						Treatment & Progress													
Case number	Race	Sex	Age	Duration	Extent	Serum electrolytes	Neutrophils	Edema	Plasma volume	Red blood cell volume	Blood volume	VOLUME packed red blood cells	Serum proteins	Total albumin	Globulin	Calcium	Inorganic phosphate	Chloride as NaCl	CO <sub>2</sub> capacity plasma	Total base excess	Remarks										
34	W	M	29	-	4	2	1	E	0	0	0	2 A	0	70	62	531	84	240	2	860	1	350	97.4	60	5.57	11.8	3.2	538	51		
33	C	F	39	-	4	4	1	D	0	0	0	0 A	0	0	59	38	135	0.2	2790	1	780	980	3	24.2	0.6	5.30	10.9	4.1	552	46	
32	C	F	25	-	12	4	1	DE	0	0	0	0 A	0	1	59	38	135	0.2	2790	1	780	980	3	24.2	0.6	5.30	10.9	4.1	552	47	
31	C	F	30	-	12	3	1	DU	0	0	0	0 A	0	0	60	39	135	0.2	2790	1	780	980	3	24.2	0.6	5.30	10.9	4.1	552	47	
30	C	F	30	-	12	3	1-3	DU	0	0	0	0 H	0	0	60	52	22	3.3	630	2	820	810	3	37.2	2.6	5.63	11.9	4.2	530	56	
29	C	F	23	-	10	3	1-3	DU	0	0	0	0 H	0	0	60	50	9	22	5.3	570	2	740	810	3	38.2	2.5	5.72	9.2	3.8	680	49
28	C	F	23	-	10	3	1-3	DU	0	0	0	0 H	0	0	60	58	6.25	0.4	350	3	250	1	900	13	14.2	7.1	5.85	10.4	4.5	550	54
27	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
26	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
25	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
24	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
23	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
22	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
21	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
20	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
19	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
18	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
17	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
16	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
15	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
14	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
13	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
12	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
11	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
10	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
9	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
8	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
7	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
6	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
5	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
4	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
3	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
2	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
1	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
0	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-1	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-2	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-3	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-4	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-5	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-6	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-7	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-8	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-9	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-10	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-11	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-12	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-13	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-14	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-15	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	
-16	C	F	23	-	10	3	1-3	DU	3	1	0	D 0	A 0	2	56	44	19	6.3	810	3	060	750	1	71.3	8.0	5.57	7.4	2.6	356	57	

**Skin lesions.** Duration: Time in weeks since appearance of skin lesions of present attack. In a number of cases before skin lesions were healed fresh ones appeared. These are referred to as recurrent skin lesions. They are considered as being a part of the same attack of miliaria as the unhealed lesions. Figures in this column do not refer to the time of appearance of recurrent lesions.

Extinct Figures in this column indicate locality where no surface area is involved.

Burned Duration	Weeks after onset	1 - 3 times a day	2 - 7 to 10 times	3 - 11 or more times a day
Seriously	1			
Occasionally	2			

Glossitis: 1 - mild diffuse redness, 2 - marked diffuse **infection** plus areas of pain, 3 - **marked infection** plus areas of ulceration. Vomiting: 0 - absent, 1 - 2 to 3 times daily, 2 - 3 to 5 times daily, 3 - **bold tongue due to a trophobly of papillae.**

Psychosis D = delusions, C = controls, S = subjects with substantial depression.

**Neuritis Symptoms** or peripheral nerves in legs, but with exaggerated knee jerks. 1 = mildly clinically subjective. 2 = moderate. 3 = severe/besideeden. — = information not available.

Gastric acid <sup>†</sup>	A = absence of free hydrochloric acid	N = normal acidity	H = hypoacidity
Neuritis	—	—	—
Syndrome	—	—	—
Diarrhea	—	—	—
Constipation	—	—	—
Flatulence	—	—	—
Bloating	—	—	—
Abdominal pain	—	—	—
Stomach cramps	—	—	—
Heartburn	—	—	—
Indigestion	—	—	—
Belching	—	—	—
Gas	—	—	—
Stomach fullness	—	—	—
Loss of appetite	—	—	—
Weight loss	—	—	—
Malnutrition	—	—	—
Malabsorption	—	—	—
Diarrhea	—	—	—
Constipation	—	—	—
Flatulence	—	—	—
Bloating	—	—	—
Abdominal pain	—	—	—
Stomach cramps	—	—	—
Heartburn	—	—	—
Indigestion	—	—	—
Belching	—	—	—
Gas	—	—	—
Stomach fullness	—	—	—
Loss of appetite	—	—	—
Weight loss	—	—	—
Malnutrition	—	—	—
Malabsorption	—	—	—

**Pituitary** (Pituitary gland). 1 — first to develop. 2 — moderate in degree and distribution. 3 — extensive.

The calculation of the hematocrit of the preceding or following study is made by comparing the hematocrit at that particular time with the hematocrit at a time when the temperature was more than 103.5° F.

The assumption that the volume of circulating blood cells is unchanged from one occasion to the next, is however, not always true.

problems of pellagra than the accumulation of more exact knowledge of the disturbed physiology

All patients studied were in-patients of Charity Hospital, and all had typical skin lesions of pellagra together with other evidences of the disease

Detailed methods will be presented as indicated in subsequent papers of this series

The first study was usually made one or two days after admission to the hospital, and with few exceptions the blood volume was determined each time blood was taken for chemical study. The studies were repeated, provided the patients remained sufficiently long under observation, at intervals of from 5 to 15 days. All patients were given during their stay in the hospital a special "pellagra diet." This diet has a daily caloric value of 2500 to 3500 calories, contains more than 100 grams of protein, a part of which is liver or sweetbread, and contains an abundance of green leafy vegetables and 500 cc buttermilk. It is given to patients with diarrhea in bland form, and, when necessary, a liquid diet of similar caloric value was given by gavage. Routinely there was included for the vitamin content 30 cc of fresh brewer's yeast three times a day, 150 cc of orange or lemon juice daily and in addition some of the patients received 4 cc of cod liver oil three times a day. The nurses usually took especial interest in their pellagrins and in their nutrition and fluid intake.

No particular effort was made to influence by treatment the derangements of physiology revealed in these studies. Therapeutic studies are to be made later. In the present studies pellagra was observed under what has been considered an excellent regimen.

The detailed clinical information, the blood volumes, and the results of chemical studies are shown for 42 cases in table 1.

The pellagrins which we have studied made a fairly representative group. The proportion of severe cases of pellagra is much greater in hospital than in general practice, but mild forms of the disease are also present.

In subsequent articles (1, 2, 3, 4) detailed procedures will be presented and the results of the physiologic investigations analyzed and discussed.

## ACKNOWLEDGMENTS

I take pleasure in acknowledging my great indebtedness to Professor John H Musser for opportunity and encouragement and to Dean C C Bass for his stimulating interest in pellagra, and to the Chiefs of Services in the Charity Hospital who encouraged me to study their patients Doctors J Birney Guthrie, Sam Hobson, S Chaillé Jamison, J A Storck, P H Jones, and B J DeLaureal

## SUMMARY OF CLINICAL DATA

Detailed clinical data obtained from a study of 42 pellagrins are recorded in one table which also includes the results of determinations of circulating blood volume, serum albumin and globulin, serum calcium and inorganic phosphorus, of plasma chlorides, of carbon dioxide combining power, and of serum total base

The group of patients consisted of 6 white men, 9 white women, 9 negro men, 16 negro women, and 2 negro children Twenty were less than 30 years old and 36 were less than 50

Thirteen patients, 31 per cent, died in the hospital

Thirty nine had had no previous attacks of pellagra, 3 had had attacks 1 to 7 years previously

Eleven patients of the group had other diseases Syphilis, 5, two with heart disease, stricture of the rectum, 4, active gonorrhea, 1, intestinal tuberculosis, 1

Fifty-nine per cent of the patients were more than 20 per cent underweight and 35 per cent more than 30 per cent underweight, and 10 per cent were overweight

Eight patients showed edema

The skin lesions at the time of the first study showed erythema in 26, 62 per cent, 9 of the 26 showed recurrent lesions in the presence of older unhealed lesions

Vomiting was a serious problem in 2 patients

Glossitis was present in 26, 62 per cent, diarrhea in 25, 60 per cent

Gastric analysis was done in 34 and achylia found in 29, 60 per cent, of the entire group, hypoacidity in 3, and a normal acidity in 2 patients

Seven patients were delirious, 2 were comatose and 1 suicidal, a total of 10, 24 per cent, with psychoses

Six patients showed evidences of peripheral neuritis

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## THE PATHOLOGIC PHYSIOLOGY OF PELLAGRA

### II THE SERUM ALBUMIN AND GLOBULIN

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The only determinations of serum protein in pellagra previously reported are those of Ballif, Rennescu and Reznic (1). They used the combined refractometric and viscometric methods in the study of 60 pellagrins. They report only the average and range of their results. The average total serum protein was 7.3 per cent, and the range from 9.3 per cent to 5 per cent. They noted a tendency to abnormally low albumin globulin ratio.

#### METHODS

Blood for analysis was drawn after an over night fast. It was collected under oil without stasis or exposure to air and without anticoagulant, and time was allowed for clotting and retraction of clot. After centrifugation the serum was pipetted off. Serum albumin and globulin were determined by Greenberg's method (2). This method employs Howe's procedure for separation of the fractions by precipitation of globulin with sodium sulphate and Wu's (3) method of colorimetric estimation. The phenol reagent of Fohn and Ciocalteu (4) was used. Values obtained by this colorimetric method agree closely with those obtained by the Kjeldahl method.

The patients were kept throughout on a daily diet described elsewhere (5) containing more than 100 grams of protein of good biological quality and rich in vitamins.

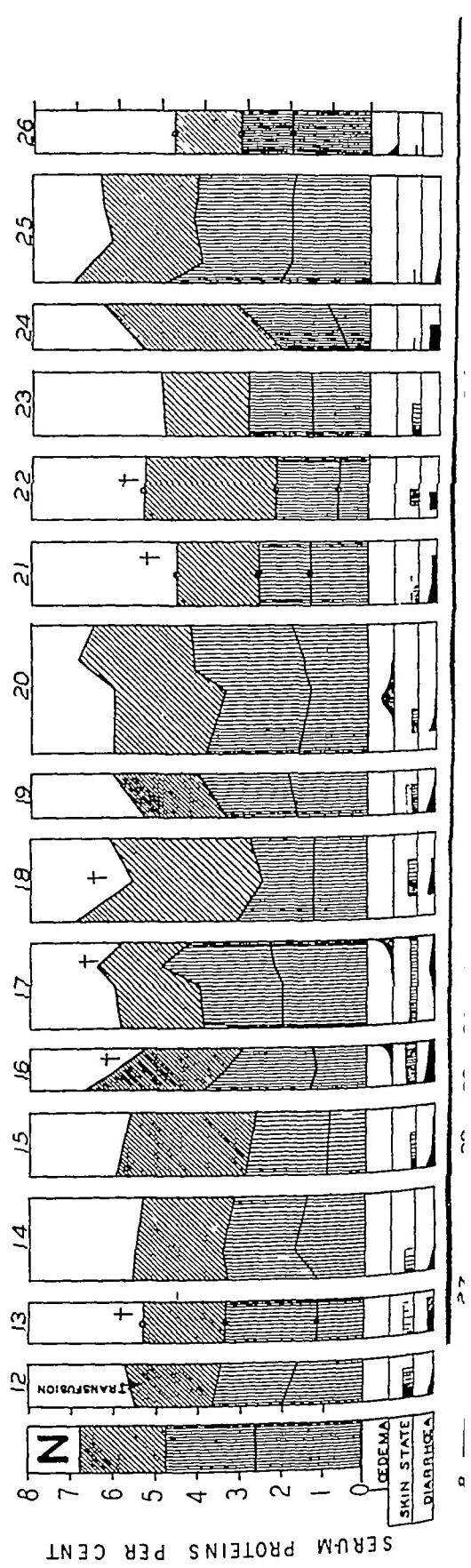
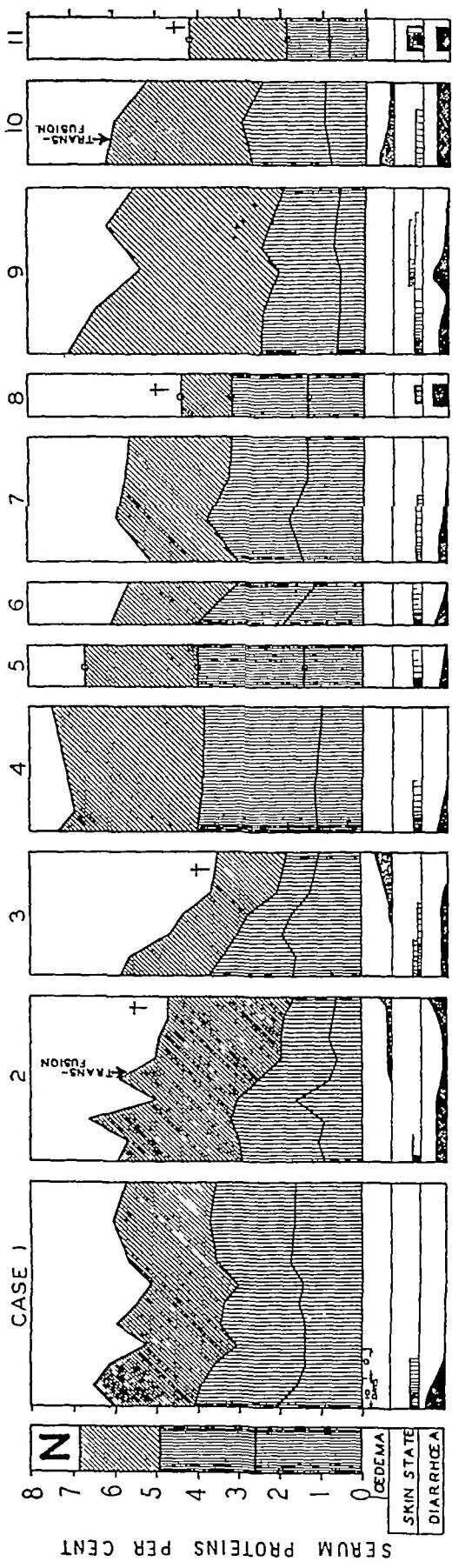
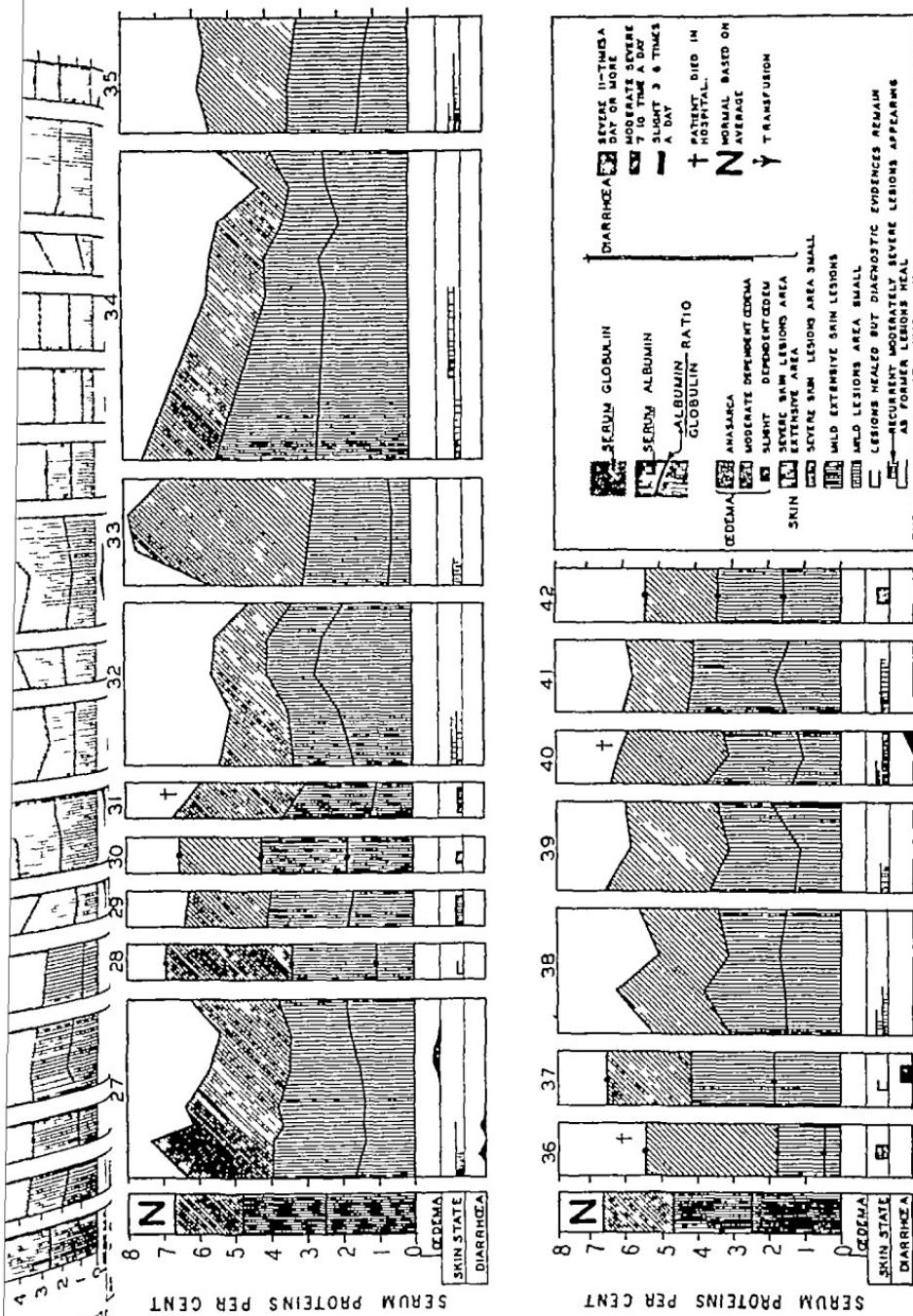


Fig 1



## RESULTS

Determinations of serum albumin and globulin were made on 129 specimens of blood from 42 cases of undoubted pellagra. The results are recorded along with other physiological data as well as the clinical information in tabular form in the first paper of this series (5). Case numbers used in this paper refer to those listed in table 1 of the first paper. The serum albumin and globulin and albumin-globulin quotients are shown graphically in figure 1. These data are further analyzed in the histograms of figure 2 and in table 1 of this paper.

TABLE 1  
*Summary of the serum albumin and globulin values*

	Pellagrins			Normal standards	
	Average	Median	Range	Average	Range
	gms per 100 cc	gms per 100 cc			
Albumin				4.80	5.65-4.20
Highest value for each patient	3.530	3.600	5.46-1.71		
Lowest value for each patient	3.035	3.093	4.22-1.64		
Globulin				1.90	2.91-1.32
Highest value for each patient	2.690	2.500	5.11-1.19		
Lowest value for each patient	2.348	2.250	4.18-1.19		
Total protein				6.70	7.65-5.60
Highest value for each patient	6.140	6.125	7.84-4.19		
Lowest value for each patient	5.433	5.430	6.85-3.42		
Albumin-globulin quotient				3.19	4.20-2.20
Highest figure for each patient	1.509	1.625	2.62-0.45		
Lowest figure for each patient	1.277	1.316	2.62-0.45		

The determination of values for a large group of healthy individuals of the same race, age, and sex distribution as the pellagrins we have studied will probably require several years. It is difficult to secure for study healthy subjects in the older age groups, particularly the colored. The values we now have agree with those reported by other authors who used methods which give values comparable to the colorimetric method. Our tentative standards for the normal serum protein values are derived largely from data by Bruckman, D'Esopo, and Peters (6) and Wiener and Wiener (7) and are shown in table 1.

*Albumin*

When only the highest albumin value for each pellagrin was considered, 4, or 10 per cent, fell within the range for normals, while 38 or 90 per cent were below the lower limit for normals. When the lowest value for each case was considered all were below the normal range, and 18 or 43 per cent were less than 3 per cent, a decidedly low value.

Changes in serum albumin content while the patient was under observation seem of particular interest. Wiener and Wiener considered 10 per cent change in an individual's serum protein level as the limit of physiologic variation. I have considered changes of greater than 12 per cent as being of possible pathologic significance. Thirty of the patients were studied more than once, 11 of these, or 27 per cent, showed no significant change in serum albumin concentration, while 14, or 47 per cent, showed decrease of more than 20 per cent, and 3, decrease of at least 40 per cent. Five patients showed definite increase in serum albumin while under study. In one (case 17) the change was undoubtedly due to dehydration from diarrhea and vomiting. For two (cases 19 and 24) the period between studies was short and the results could be partially explained by changes in plasma volume. Due to the fact that the patients left the hospital before other studies could be made, it is not known whether the gains were more than temporary. However, there was some indication of a return to normal. In another patient of this group (case 32), after a month of gradual gain in serum albumin, there was a definite loss. In only one patient (case 20) was there a return to approximately normal serum albumin values in association with striking clinical improvement.

The graph in figure 3 represents an effort to investigate correlation between the state of nutrition and the serum albumin concentration. For each patient the percentage deviation from ideal body weight is plotted against the percentage deviation from average normal serum albumin values. The lowest weight and the lowest figure for albumin for the period the patient was being studied were used. Serum albumin concentration was not always lowest for a given patient when the body weight was at its lowest. The appearance of edema sometimes caused body weight to rise even though serum albumin fell. The in-

terval between the time of lowest body weight and the time of lowest serum albumin concentration was never more than three weeks and usually only a few days Standard for ideal weights were those of Tables IV and IX of "Medico Actuary Mortality Investigations" according to sex, age, and height These tables, of course, give average weights, but may be used satisfactorily for determining for a given patient the ideal weight Inspection of the spot graph shows for the whole group doubtful correlation between serum albumin and fall of weight However, for a considerable number of the patients there appears to be correlation of some importance, the exceptions fall into interesting groups Of those whose body weights were higher in proportion to serum albumin level, three (cases 38, 39, and 14) were characterized by the absence or mildness of gastro-intestinal symptoms One (case 10) had ascites due to tuberculous peritonitis and edema of the extremities associated with severe anemia In three (cases 40, 11, 36) the disease was fulminating and rapidly fatal These instances suggest that fall in serum albumin may be a more sensitive index of disturbed nutrition in pellagra than is loss of body weight Two (cases 9, 33) showed high globulin concentrations There were fewer patients whose body weights were proportionately lower than their serum albumin levels One (case 4) had syphilis, another (case 25) was not studied until after all major symptoms of pellagra had disappeared When these eleven exceptions are excluded from the group, those remaining are too few to justify statistical analysis For the whole group the coefficient of regression of weight on serum albumin was  $0.26 \pm 0.16$

The relationship of serum protein concentration to edema in the group of pellagrins was not clear One patient (case 3) developed slight generalized edema when the serum proteins fell to very low levels and another (case 2), with almost equally low serum proteins, showed only slight dependent edema The latter suffered from diarrhea Three patients (cases 21, 23, 11) showed no edema in spite of low serum proteins Four patients (cases 9, 15, 18, 22) who had no edema had serum albumin values of less than 3 per cent, but total proteins were above 5 per cent One patient (case 10) with ascites and tuberculous peritonitis showed marked edema when the serum albumin was between 2.50 per cent and 3 per cent and the total proteins

5 per cent or 6 per cent. In this case there was an extremely high plasma volume and a very low hematocrit reading. The patient suffered from dyspnea on slight exertion. Three patients (cases 16, 17, 20) showed edema when the serum proteins were practically normal. Two of these (cases 16, 17) showed marked oliguria due to diarrhea and vomiting, and edema appeared a short time before death. One patient (case 26) was so edematous five days before our study that unwarranted diagnosis of congestive heart failure was made although edema had disappeared at the time of our study. Her serum albumin was 3.16 per cent and total proteins 4.75 per cent.

#### *Diarrhea*

For 26 patients (cases 1 to 26) diarrhea was a symptom of considerable importance, while in the remaining 16 (cases 27 to 42) there was no diarrhea or it was either mild or of brief duration. Considering only the lowest serum albumin value for each patient, the average for the group with diarrhea was 5 per cent lower, and the average for the group without diarrhea 8.1 per cent higher, than the corresponding general average for the whole group of 42 pellagrins. When only high albumin values were considered the differences between averages was in the same direction but of less magnitude.

The value of serum albumin concentration as an index of severity of pellagra may be judged by examining the values for the fatal cases. Considering those who died within three weeks after study, and were not known to suffer from heart disease and were not dehydrated, there were 9 cases. The average for their lowest serum albumin was 2.20 per cent as compared to 3.03 per cent, the corresponding figure for the group as a whole.

#### *Globulin*

The histogram in figure 1 shows the tendency to high serum globulin values in our group of pellagrins. When only the highest value for serum globulin for each patient was considered, only three, 7 per cent, were less than 1.90 per cent, which is an average value for normal globulin, and when the lowest value for each patient was considered, there were only 9, 21 per cent, with figures 1.90 per cent or less.

There were 13 patients in the group whose highest globulin values

were greater than 3 per cent. Two had luetic heart disease (cases 18 and 31) another (case 4) a positive Wassermann reaction without clinical evidence of syphilis, four (cases 2, 24, 28, 32) had stricture of

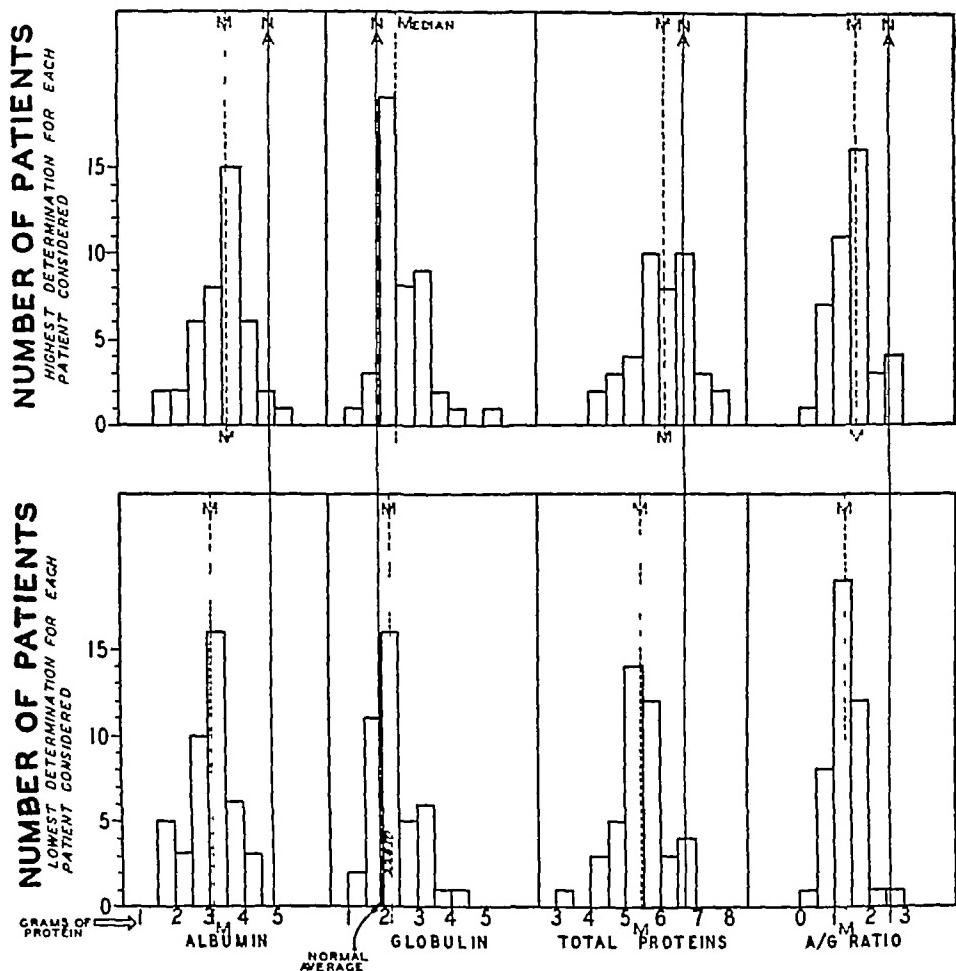


FIG 2 HISTOGRAMS SHOWING DISTRIBUTION OF VALUES FOR SERUM ALBUMIN, SERUM GLOBULIN AND ALBUMIN-GLOBULIN RATIO

the rectum. One patient of the 13 suffered from intestinal tuberculosis (case 10), and another had active gonorrhea (case 9). Four of the 13 patients (cases 15, 36, 48, and 22) had no complications, but had such severe skin damage as to involve tissue necrosis. When one

excludes the globulin values for the above mentioned 9 patients with syphilis, tuberculosis, gonorrhea and stricture of the rectum, and the values for two other cases of syphilis, the tendency toward high globulin values becomes much less pronounced. The average of the highest values for the 31 uncomplicated cases was 2415 as compared to 2690 for the entire group of 42. The median was 2324 as compared with 2500.

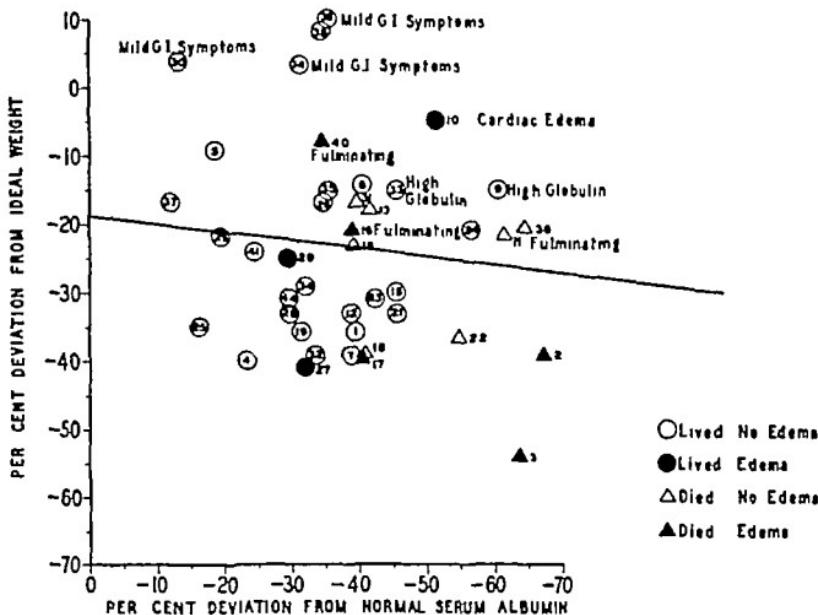


FIG. 3 SPOT GRAPH SHOWING RELATIONSHIP BETWEEN UNDERWEIGHT AND CONCENTRATION OF SERUM ALBUMIN, BASED ON LOWEST SINGLE WEIGHT AND LOWEST SERUM ALBUMIN VALUE FOR EACH PATIENT  
Line shows regression of weight on serum albumin

The concentration of serum globulin in a given individual tended to be much more constant from week to week than that of albumin. Three patients (cases 28, 32, 34) showed irregular fluctuation in globulin concentration. It may be of some interest that they suffered from neuritis. Two patients (cases 33, 35) improved clinically as serum albumin values gradually fell and the globulin concentration

increased One patient (case 17) died soon after a definite drop in globulin Another patient (case 2) began a steady decline clinically and in serum albumin concentration immediately after a brief period of decreased globulin concentration

### *Total Proteins*

As indicated by the histograms in figure 2, total serum proteins, that is albumin plus globulin, tended to be low in our group but less strikingly so than albumin, due to the many high figures for serum globulin Considering only the highest value for each case, there were 11, 26 per cent, with total serum protein concentration of 6.70 per cent or more while 31, or 74 per cent, were less than this normal average However, 31, or 74 per cent, of the 42 cases showed total protein values in the normal range, that is 5.60 per cent or greater When only the lowest value for each case was considered all but two, 95 per cent, were below the normal averages, 15, or 36 per cent, were within the range for normal and 27, or 64 per cent, were below 5.60 per cent, the lower limit for normals

### DISCUSSION

Our knowledge of the physiology of the serum proteins is still extremely meagre, and any explanation given at present for abnormal serum protein levels is based on incomplete evidence However, the work of Kohman (8), Maver (9), and Frisch, Mendel and Peters (10), on experimental animals has shown that protein starvation produces a reduction in serum proteins Low serum proteins were found in patients suffering from malnutritional edema by Schittenhelm and Schlecht (11) and by Jansen (12) Bruckman, D'Esopo and Peters (6) studied the serum proteins in poorly nourished patients with diabetes, with obstructions of the gastro-intestinal tract, and with chronic pulmonary infections and found low values Wiener and Wiener (7) found low albumin values in patients with a variety of infections Their report does not mention the state of nutrition

It has now become well known that there exists, in certain types of nephritis, lowering of serum proteins, especially of albumin This is especially true of those patients who suffer from prolonged, marked albuminuria Repeated examinations of the urine for albumin in our

cases excluded albuminuria as the mechanism responsible for low serum albumin.

Many observers have been impressed with the faulty or inadequate protein of the pellagrin's dietary. It has been considered by many as the chief factor in the pathogenesis of the disease. If protein starvation were a major cause of the disease, one would expect to find low serum albumin in the early stages of pellagra. The patients who were studied in the very early stages of the first attack showed values nearly normal. This observation in addition to the observed tendency for serum albumin to fall after the diagnostic evidences of pellagra had disappeared, has led me to suspect that the low serum albumin concentration was a result of pellagra, and represents impaired digestion or absorption of protein, and is dependent upon injury to the alimentary tract by pellagra. It is a well known fact that damage to the alimentary tract in pellagra is a frequent if not invariable occurrence. It may involve any portion of the alimentary mucosa from lips to anus, and has been described histologically by Denton (13) as degenerative in character. This injury is manifested clinically by glossitis or stomatitis, by dysphagia and esophageal pain, by vomitus streaked with blood in certain very severe cases, by diarrhea and abdominal discomfort and by injection of the rectal mucosa as viewed through the proctoscope. Many observers have been impressed by the idea that, in a given patient, pellagra may involve, predominantly, only one system, whether this be the skin, alimentary tract or nervous system, and causes little or no damage to the other systems. When the alimentary tract of pellagrins, who died during the earlier stages of the disease, was examined at autopsy the distribution of injury, as indicated by areas of injection, superficial erosion or ulceration, was found to be quite irregular. In one instance the mouth and esophagus and lower colon showed the greatest damage, while in another the upper small intestine was chiefly involved. Even in a given portion of the small intestine itself, the injury seems to involve irregular patches of several square centimeters in area, leaving others apparently undamaged. The value of the available histological studies of the alimentary tract in pellagra is impaired by the possibility of postmortem auto digestion, by an insufficient variety of cases, and by inadequate correlation with clinical study. The pathologist reported extreme

atrophy of the mucosa of the small intestine for the pellagrin, (case 3) who, in spite of taking an excellent diet, was the most striking example of undernutrition

It is evident from the work of others, already cited, that low serum albumin is to be found in many conditions I have studied a variety of gastro-intestinal conditions, especially those with diarrhea as a chief symptom, and have found low serum albumin in a number Serum albumin may fall rapidly in acute bacillary dysentery, but it is striking how rapidly it may return to normal with termination of the disease In acute dysentery it is easy to imagine that there is a considerable loss of serum protein from the intestinal mucosa as a part of the exudative inflammatory processes This may also happen in pellagra during the stage of acute damage to the alimentary tract Three cases of chronic moderately severe diarrhea, apparently non-pellagrous, were found in which there were normal serum proteins

It is possible that injury to the pancreas may occur in pellagra resulting in deficient secretion of the proteolytic enzymes In another article of this series (14), evidence is presented to show that in pellagra serum electrolytes are frequently diminished This probably represents excessive loss of electrolytes in the feces Excessive secretion of pancreatic juice may be involved, followed by a period of hypofunction

These data suggest that, in many instances, the dependent edema of pellagra is due to decreased colloid osmotic pressure of the blood plasma Goldberger and his co-workers (15) considered the edema in their group of pellagrins, in the Georgia Insane Hospital, as evidence of beriberi The edema of beriberi is not well understood and may itself be due partly to low serum proteins Most of our patients had been taking abundant antineuritic vitamin Why some pellagrins did not develop edema in spite of low serum albumin is not known Low hydrostatic pressure in the capillaries is one possible explanation, since there was definite arterial hypotension in these particular patients as well as in nearly all the other pellagrins Another possible factor is the ease with which water escapes from the serum into the lumen of the gut in association with diarrhea Low electrolyte content of the tissues may be of importance

The very low serum albumin in the patients who died of pellagra,

and the relatively normal values in those with mild pellagra indicate that determinations of serum albumin are of importance as furnishing an index of severity of the disease, and as a basis for prognosis.

Is one justified in demanding that serum albumin return to normal before classifying the patient as cured? If my suggestion that the pellagrin's inability to build up serum albumin to a normal level is due to damaged digestion, is supported by further work, then the situation of a pellagrin suffering from malnutrition, in spite of adequate diet, will be similar to that of a patient who has passed through one attack of acute nephritis and then suffers from impaired kidney function.

#### SUMMARY

Determinations of serum albumin and globulin are reported for 129 specimens of blood from 42 pellagrins and the results analyzed in table, graph and histograms.

Every patient, at some time during the period of study, had a serum albumin value below 4.20 per cent, and 95 per cent of the patients had at least one value less than 3 per cent.

There was little tendency for serum albumin concentration to return to normal, and frequently there was a considerable decrease during the consumption of a diet adequate in protein and vitamins.

Two patients died long after characteristic evidences of pellagra had disappeared, and in spite of taking an excellent diet, with a serum protein content similar to that seen in protein starvation. The mechanical effects of diarrhea on digestion did not seem to constitute a major factor in the chronic malnutrition observed.

For part of the group, the decrease in concentration of serum albumin seemed to be proportional to the degree of undernutrition as indicated by body weight. Exceptions were chiefly individuals whose body weights were higher for their serum albumin values than the remainder of the group and who had very mild gastro-intestinal symptoms or fulminating rapidly fatal pellagra or edema, or whose serum globulin values were high.

Serum globulin concentration tended to be slightly increased in the patients with uncomplicated pellagra, except in the patients with skin necrosis, certain chronic infections and stricture of the rectum, who showed moderate increase.

Total serum protein concentration tended to be low but not as low, relatively, as serum albumin

#### CONCLUSIONS

In pellagrins there is a tendency toward low serum albumin concentration, which seems to develop after the disease is manifest and frequently remains long after the diagnostic evidences of the disease have disappeared and in spite of adequate diet

It is suggested that the abnormality is one of disturbed digestion, causing difficulty in the absorption of proteins, dependent upon injury of the digestive system by pellagra

Serum protein determinations are of value in estimating the severity of pellagra and in furnishing one criterion of cure

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## THE PATHOLOGIC PHYSIOLOGY OF PELLAGRA

### III THE SERUM CALCIUM AND PHOSPHORUS, WITH ESPECIAL REFERENCE TO NERVOUS SYMPTOMS

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Interest in the calcium and phosphorus metabolism of pellagra was based chiefly upon the clinical observation that sunshine is injurious to the pellagrin. This caused the disease to be compared with rickets, which is prevented or cured by sunshine. The two diseases afford a number of interesting contrasts. Rickets is a disease of the period of most rapid growth, particularly during infancy, early childhood and adolescence, while these are the age periods showing the lowest incidence of pellagra (1). Rickets develops in the winter and early spring and disappears during the summer, while pellagra appears in the late spring and summer and practically disappears in winter. Rickets is a disease of cities, and pellagra of the rural districts. Childbearing and lactation seem to be predisposing causes for both pellagra and osteomalacia, which may be considered a form of rickets. As far as injurious effect of light is concerned the relation to pellagra seems to be chiefly with lactation rather than pregnancy. Siler, Garrison, and MacNeal (2) brought out statistical evidence to show that, in pellagra, the period of gestation is a period of resistance to pellagra, as far as the manifestation of erythema is concerned. They also showed that the first three months after childbirth might be a time of great susceptibility to the erythema of pellagra for the mother, particularly if delivery occurred during the first eight months of the year. If childbirth occurred during the last four months of the year, the mother was particularly resistant to pellagra for the next four months. In-

stead of gestation being a period of lessened susceptibility to osteomalacia it is a period of great danger (3)

Denton (4) found histological evidence of beginning arteriosclerosis in young pellagrins Many workers (5) have shown that an excess of vitamin D in the form of irradiated ergosterol promoted arteriosclerotic processes The dietary, which is supposed to cause pellagra in the Southern United States, is rich in unirradiated ergosterol taken with pork fat or cotton seed oil The evidence just cited suggested that with the coming of the spring sunshine the ergosterol in the individual's skin might be activated in sufficient quantity to provide an excess of vitamin D From another viewpoint the physiology of calcium might be of interest in pellagra Much has been written about the influence of calcium on permeability of cell membranes It is likely that the skin lesions of pellagra involve increased permeability of capillaries While determinations of serum calcium and inorganic phosphorus may detect only the more gross abnormalities of metabolism, they may be of great value, particularly for orientation

Ballif and Gherscovici (6) report that they determined the serum calcium by the method of Ward for 10 patients with severe pellagra, and that they constantly found values above the normal 10.5 to 13.5 mgm per 100 cubic centimeters They comment upon the unexpected occurrence of "hypercalcemia" combined with exaggerated reflexes

#### METHODS AND MATERIAL

Venous blood was collected, without stasis, after a night's fast and allowed to clot under oil Calcium was determined by Clark and Collip's modification (7) of the Kramer and Tisdall method A fine capillary tip was fitted to the micro burette which delivered small drops of permanganate solution Inorganic phosphate was determined by Gunther and Greenberg's (8) modification of the method of Fiske and Subbarow Parallel determinations by the method of Fiske and Subbarow and by Greenberg's modification gave excellent agreement The methods for determining serum albumin and globulin have been presented in a preceding article (9)

The patients studied for this report consist of 42 pellagrins, described in tabular form in article I of the present series (10), 6 additional patients with typical pellagra too incompletely studied to include in the

TABLE I

Data for patients other than the 42 pellagrins described in Article I of the present series of papers

Case number	Calcium deviated from calculated value per cent	Serum calcium mgm. per 100 cc.	Serum inorganic phosphorus mgm. per 100 cc.	Albumin gm. per 100 cc.	Serum globulin gm. per 100 cc.	Serum albumin + globulin gm. per 100 cc.	Diagnosis
43	+12	11.8	4.7	7.05	1.39	8.44	Early pellagra—vomiting and diarrhea. Dehydration. Died
44	+16	10.7	4.4	3.64	2.42	6.06	Moderately severe pellagra, skin lesions 1 month
45	+15	11.7	3.4	5.03	2.09	7.12	Convalescent pellagra, serum albumin previously very low
46 {	+42	10.7	13.3	4.13	2.94	7.07	Severe pellagra, skin necrosis, vomiting, diarrhea, dehydration, died
46 {	+33	10.1	11.6	3.88	2.50	6.38	
47	-5	8.8	3.5	3.42	2.34	5.76	Early pellagra. Carcinoma of cervix, with metastasis, radium therapy, died
48 {	+15	10.3	4.3	3.86	1.67	5.53	Convalescent pellagra having shown great clinical improvement before study
48 {	+11	10.1	4.5	4.11	1.76	5.87	

## Pellagra-like conditions

49	+24	11.5	5.4	4.19	1.72	5.91	Glossitis
50	+23	10.5	3.9	3.76	0.85	4.61	Diarrhea. Glossitis
51	+7	10.8	3.2	5.05	2.04	7.09	Diarrhea, achylia. Atypical skin lesions
52 {	-7	9.10	5.4	4.33	3.08	7.41	Recent diarrhea glossitis
	0	9.9	2.9	3.93	2.62	6.55	
53 {	+18	10.8	5.5	4.07	2.27	6.34	Glossitis, loss of strength and weight
	+7	10.2	4.5	4.25	2.40	6.55	
54 {	+24	11.6	4.6	4.18	2.18	6.36	
	+14	10.4	4.8	3.42	2.63	6.05	Diarrhea ptyalism, glossitis
54 {	+14	10.6	4.1	3.40	2.54	5.94	
	+9	10.2	4.5	3.52	2.74	6.26	
55	+24	11.0	4.2	3.79	1.57	5.36	Definite sunburn, ptyalism, achylia

## Definite bone disease

56	+41	13.2	3.4	2.74	3.82	6.56	Multiple myeloma
57 {	+10	11.2	3.2	4.53	2.60	7.13	Luetic periostitis
	+18	10.5	4.1	4.27	1.19	5.46	
58	+12	10.4	4.5	3.22	2.96	6.18	Luetic periostitis

TABLE 1—Concluded

Case number	Calcium deviated from calculated value	Serum calcium	Serum inorganic phosphorus	Albumin	Serum globulin	Serum albumin + globulin	Diagnosis
Patients with various diseases							
59	-29	7.9	3.3	7.65	1.30	8.95	Luetic heart disease, edema
60	+13	10.0	5.1	3.84	1.88	5.72	Arsenical dermatitis
61	+10	10.0	5	3.52	2.48	6.00	Arteriosclerotic heart disease, edema
62	+11	9.6	4.8	4.43	0.77	5.20	Pernicious anemia
	+9	10.3	4.3	5.03	1.28	6.31	
63	+2	8.8	4.5	3.73	1.21	4.94	Pernicious anemia
64	+12	10.2	3.8	3.58	2.03	5.61	Luetic and hypertensive heart disease
65	+6	9.9	3.9	3.52	2.50	6.02	Gastrogenous diarrhea
66	+8	10.3	3.4	5.05	1.18	6.23	Healthy middle aged adult
67	-4	9.7	4.1	4.76	2.71	7.47	Menopause
68	-11	8.6	3.2	3.85	2.42	6.27	Pulmonary tuberculosis Syphilis
69	+8	10.9	4.6	5.31	2.32	7.63	Obesity Pyelitis
70	-2	10.2	5.3	4.52	4.01	8.53	Salpingitis Pulmonary tuberculosis
71	0	10.0	4.7	5.07	2.55	7.62	Neuritic beriberi
	-13	10.6	3.8	4.02	2.03	6.05	
72	-6	9.8	4.6	4.43	3.68	8.11	Salpingitis
73	-3	9.3	3.8	3.80	2.71	6.51	Hypertensive heart disease
74	+11	10.8	4.7	3.50	1.46	4.96	Early gastric cancer
	+14	10.3	3.7	3.80	1.59	5.39	Bacillary dysentery
75	+15	9.2	4.4	3.76	1.52	5.28	
	0	10.3	4.4	4.28	3.68	7.96	
76	+6	9.8	3.4	3.60	1.98	5.58	Amebic dysentery
77	+5	9.3	3.9	3.37	1.84	5.21	Bacillary dysentery
78	+6	9.7	3.1	3.03	2.24	5.27	Cancer of lung
79	+4	9.5	2.9	3.60	1.59	5.19	Hemiplegia, fecal incontinence, mild chronic diarrhea
80	+12	10.8	4.5	4.42	1.40	5.82	Diarrhea, chronic
81	+22	10.6	3.1	2.85	3.44	6.29	Pernicious anemia
82	+2	9.2	2.9	2.10	2.82	4.92	Bacillary dysentery
	+2	9.1	2.9	2.26	2.41	4.67	

general study, 7 patients suffering with disorders clinically like pellagra but without typical skin lesions, 3 patients with known disease of bones and 24 patients clinically without pellagra but suffering from a wide variety of diseases and without clinical evidences of disturbed

calcium metabolism. In all, 181 simultaneous determinations of serum calcium, inorganic phosphorus, albumin and globulin were made on 82 individuals. The figures for individual determinations for 42 of the pellagrins are recorded elsewhere (10) and for all other patients in table 1.

Peters and Eiserson (11) derived a formula for determining expected serum calcium concentration which applies to the serum of individuals with normal calcium metabolism when total serum proteins (albumin and globulin) and inorganic phosphorus are known. Their formula is  $Ca = 0.255 P + 0.556 \text{ protein} + 7$ , when calcium and phosphorus are expressed in milligrams per 100 cc and protein as grams per 100 cc. The mode of presentation of our data is based on their formula. Throughout this paper attention will be directed toward abnormalities in serum calcium levels, although it is realized that the phosphorus level may be equally or even more at fault. It may be noted, however, from the absolute values given in the tables that usually it is calcium and not phosphorus which deviates most from the usual mean values.

In figure 1 the results of the determinations of serum calcium for each patient are shown as per cent deviation from the calculated value, using the formula of Peters and Eiserson. By inspection of this graph it will be noted that there was a tendency, in the group of pellagrins, for serum calcium levels to be considerably higher than the calculated values, while the determined serum calcium content for patients without pellagra and without obvious bone disease approximated very nearly the calculated figures. One patient (case 56) suffering from extensive bone absorption due to multiple myeloma showed a serum calcium content 42 per cent above the calculated level, while 2 patients with luetic periostitis showed only slight increases.

More striking than the contrast between serum calcium levels of the patients with and without pellagra is the contrast between the serum calcium levels of individual pellagrins and groups of pellagrins. It will be noted that certain patients with pellagra showed persistently high serum calcium, others were persistently near expected, or normal levels, while considerable fluctuations are noted for others. When the patients are grouped according to the degree of deviation from the calculated calcium level, certain clinical features are found to be of interest.

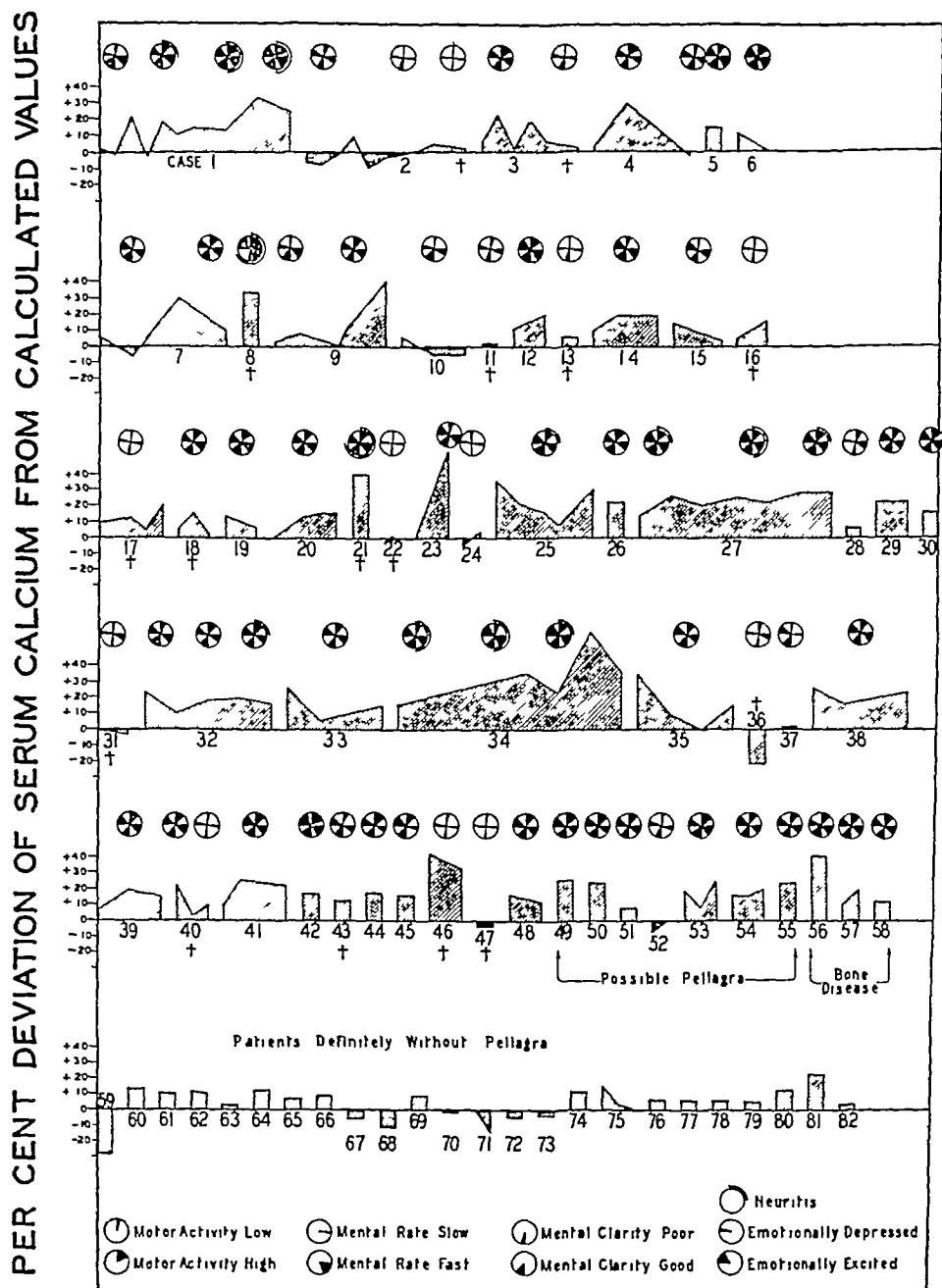


FIG. 1

The patients who died in the hospital, indicated by crosses in figure 1, for the most part showed little deviation of their serum calcium from calculated values. This is true of two other patients (cases 10 and 24), whose clinical condition at the time of discharge was such as to make death very likely within a week or two. However, there are striking exceptions. Three patients (cases 8, 21, 46) whose serum calcium concentrations were abnormally high died. Two of these patients (cases 8, 21) are of interest because of the unusual nervous manifestations which will be discussed later. The other (case 46) showed very high serum phosphorus concentration, probably due to oliguria. The patient suffered from both vomiting and diarrhea.

The mental status of pellagrins is of great importance, and should be an inviting field for psychiatric investigation. The so-called dementia has been much stressed in clinical teaching. It is an unfortunate term. The psychoses occurring in pellagra form several entities and not a single one. They include delirium, stupor, coma and the manic-depressive psychoses. In addition to the outspoken major psychoses, many pellagrins show depression without psychosis, apathy, a tendency to excessive sleep or insomnia and frequently extreme neurasthenia. In reviewing the psychic states of the pellagrins of our group we have been struck by a rough relationship between serum calcium levels and mental patterns. This relationship is obvious only when certain clinical extremes are considered. The patients who died while under observation were, with few exceptions, those who showed striking mental abnormalities, such as delirium, coma or stupor, and it is just these whose serum calcium concentrations are nearest to calculated values. The exceptions are three (cases 8, 21 and 46). It is worthy of note that the first of these exceptions (case 8), with abnormally high calcium, showed the rather unusual type of delirium for pellagra was overactive, requiring restraint. He was a drunkard, but his psychosis was not clearly either an hallucinosis or delirium tremens. The second (case 21) suffered for more than a month from severe peripheral neuritis, showed ankle clonus, but was mentally clear and quick at the time of our study. Shortly after, she developed hyperpyrexia without known cause, became delirious, talked continuously and died. Ten others of the 16 pellagrins (cases 2, 11, 13, 16, 17, 22, 31, 36, 40 and 47) who died showed psychoses characterized by hypo- rather than

hyperactivity, mental and physical, varying from drowsiness, with periods of confusion and disorientation, to coma. None of these ten showed a serum calcium content which varied from the calculated value by more than 11 per cent and the average deviation from the calculated values is +2 per cent. Another pellagrin (case 37), though without psychosis, showed such apathy and mental retardation that a clinical diagnosis of hypothyroidism was made. Of two determinations of basal metabolic rate one was low and the other normal. Contrasting clinically with these hypoactive pellagrins, the most extreme examples of which are the ten patients just mentioned, were individuals who without psychosis tended to be overactive physically and mentally, who were out of bed on every possible occasion, were quick to answer questions, frequently were witty and were considered the life of the ward. Six patients (cases 1, 21, 25, 27, 32, 34) were of this type. They all showed, as compared with calculated values, high serum calcium content, the average deviation from calculated values being +24 per cent. Each of these six patients also showed another clinical feature—evidence of peripheral neuritis and also of cord damage. They complained of severe pains in the legs and less severe pains in the hands for long periods. The calf muscles were tender and there were areas of impaired sensation for pain over the tibia, while the deep reflexes were greatly exaggerated. One patient (case 21) showed an ankle clonus. No patients other than these showed clear evidence of neuritis, though it may have been overlooked in some of the psychotic patients. Two of these patients (cases 1 and 34) had been heavy drinkers of alcohol, one (case 34) recently enough for it to have played an important part in the pathogenesis of the neuritis. The peculiar neuritis seen in pellagra is believed, by some observers, to be due to arsenic or to complicating beriberi. The patients here referred to were scarcely improved by a diet containing an abundance of anti-neuritic vitamin, and they gave no history of taking arsenicals.

Between these two extremes clinically and chemically fall the majority of our pellagrins.

As to the stage of the disease represented by the high and normal calcium groups there seems to be little difference. The normal calcium group contains a greater number of early cases than the other. Final interpretation of our observations on serum calcium must await further

study, directed particularly to determinations of calcium intake and output. From serum calcium determinations alone it is impossible to say whether high levels for calcium mean positive or negative calcium balance. In a general way the findings may be said to indicate that there is in pellagra some influence which tends to give elevated serum calcium levels, and that in the very severe cases there is another influence which tends to bring serum calcium back to normal levels. The first factor might be either excess of vitamin D, which tends to promote increased absorption and deposition of calcium in the tissues, or a factor like hyperparathyroidism, which causes excessive excretion of calcium. Differences in hydrogen ion concentration apparently are not responsible for differences in serum calcium concentration since the CO<sub>2</sub> combining power in nearly all instances was within normal range, and in those in which it was abnormal there was no definite peculiarity in regard to serum calcium concentration.

The possibility that albumin and globulin might differ in their effect on the solubility of calcium was entertained. The formula of Peters and Eiserson assumes that they have equal value. Instances were found in the data which seemed to indicate such a difference, but they were too few to carry much weight.

At first glance one suspects that the reason serum calcium levels are lower in severe pellagra than in milder forms of the disease, is that fixed base, of which calcium is one constituent, is low. This idea receives some support from studies of correlation. It was found that slight correlation<sup>1</sup> existed between per cent deviation of calcium from calculated value and per cent deviation of total base from 155 m Eq per liter.

The diet taken by our pellagrins while under observation was fairly rich in vitamin D. Ninety cubic centimeters daily of fresh unirradiated brewer's yeast and 100 grams of liver were the chief sources of vitamin D. A few patients received small doses of cod liver oil, but serum calcium was not apparently affected in those cases.

Havard and Hoyle (12) found that doses of irradiated ergosterol which were active therapeutically for rickets, were without effect on the serum calcium and phosphorus of an healthy adult, while Kam-

<sup>1</sup> $r = +0.398 \pm 0.08$

insky and Davidson (13) noted marked rises in serum calcium in tuberculous adults with similar dosage. It is very likely that various states of health may cause individuals to react differently to vitamin D therapy. It is of some interest that the highest serum calcium level for one patient (case 34) followed prolonged daily exposure, while dressed, to the rays from the summer sky. Only one other patient (case 1) was exposed to more light than the diffuse light of the ward. During the latter one-half of the time he was under study he exposed himself dressed in pajamas to the radiation from the sky for from one to three hours daily.

It is possible that the failure of calcium to reach the higher levels in the severe cases, is due to a severely damaged intestinal mucosa which interferes with absorption. Studies on healthy individuals indicate that serum calcium levels seem to be above the calculated normal more frequently than below it. Another factor which may be of importance is the reaction of the intestinal contents, which is known to have an influence on the absorption of calcium and phosphorus. The frequent achlorhydria of pellagra suggests that there is in many cases a change in reaction of the intestinal contents in the direction of alkalinity. The few patients in our series whose gastric contents contained free hydrochloric acid were not uniformly different from those showing achlorhydria.

Diarrhea was more usually associated with the lower serum calcium concentrations and may have interfered mechanically with calcium absorption. If intestinal damage were the chief factor in regulating serum calcium levels for pellagrins on complete diet, the relationship between nervous symptoms and serum calcium might prove to be incidental and of minor importance.

#### SUMMARY

One hundred and eighty-one simultaneous determinations of serum calcium, inorganic phosphorus, albumin, and globulin are reported in a study of 48 pellagrins, 7 patients with pellagra-like conditions, 24 ward patients with heterogeneous disorders and 3 patients with bone disease.

Observed values for serum calcium were compared with the calculated values based on concentration of inorganic phosphorus and serum

protein (albumin plus globulin) using the formula of Peters and Eiser-son. Per cent deviation from calculated values for each determination is indicated graphically.

As a group, the pellagrins showed a tendency for observed values to be higher than the calculated figures, whereas the patients without pellagra showed little difference between calculated and determined values.

The pellagrins with little deviation of the observed values for serum calcium from the calculated values, were characterized clinically by psychoses and fatal terminations. The psychoses of this group were marked by hypoactivity, mental and physical.

Six pellagrins had determined serum calcium concentrations markedly greater than the calculated values and were characterized clinically by tendency to mental and physical overactivity, conspicuous clearness of mind and also by evidences of neuritis. One patient with high serum calcium presented wild delirium.

The majority of the patients with pellagra were grouped on clinical grounds and according to their serum calcium levels in an intermediate position between the extremes mentioned above.

Calculation of correlation coefficient indicates that serum calcium levels are slightly correlated with concentrations of total fixed base.

#### CONCLUSIONS

Sufficient evidence of disturbed serum calcium concentration is presented to justify further study.

Abnormalities of serum calcium concentration in pellagrins seem to be related to disturbances of the nervous system. Whether the relationship is causal or incidental is unknown.

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# THE PATHOLOGIC PHYSIOLOGY OF PELLAGRA

## IV SERUM ELECTROLYTES AND ACID BASE EQUILIBRIUM

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Many clinical observers have considered acidosis to be a common and important feature of pellagra, an impression due chiefly to the acid reaction of the urine. An elaborate theory, based largely on an active imagination, explained the pathogenesis of the disease as an acidosis due to taking acid silicates in the drinking water. The first clear indication that a disturbance in acid base equilibrium occurred in pellagra was furnished by Sullivan and Stanton (1), who showed that for some pellagrins an abnormally high proportion of the urinary nitrogen was bound as ammonia.

Ballif and Gherscovici, in Roumania, are the only workers who have used modern methods in the study of acid base equilibrium in pellagra. Their reports are characterized by a distressing scarcity of clinical and chemical detail. They report (2) determinations of CO<sub>2</sub> combining power for 80 pellagrins by stating that for 59 per cent it was less than 65 volumes per cent, for 27 per cent less than 50 volumes per cent, for 6 per cent less than 40 volumes per cent, and for 8 per cent greater than 65 volumes per cent. They also found that the administration by mouth of 10 grams of sodium bicarbonate had little effect on the urinary pH and the CO<sub>2</sub> combining power of the plasma. In a later study (3) they determined plasma pH by a colorimetric method, and for 20 patients with grave acute pellagra they found an average pH of 7.27, and for the same patients an average CO<sub>2</sub> combining power of 49 volumes per cent. For 10 patients with chronic pellagra the average values given were normal. Ballif, Rennescu and Reznic (4)

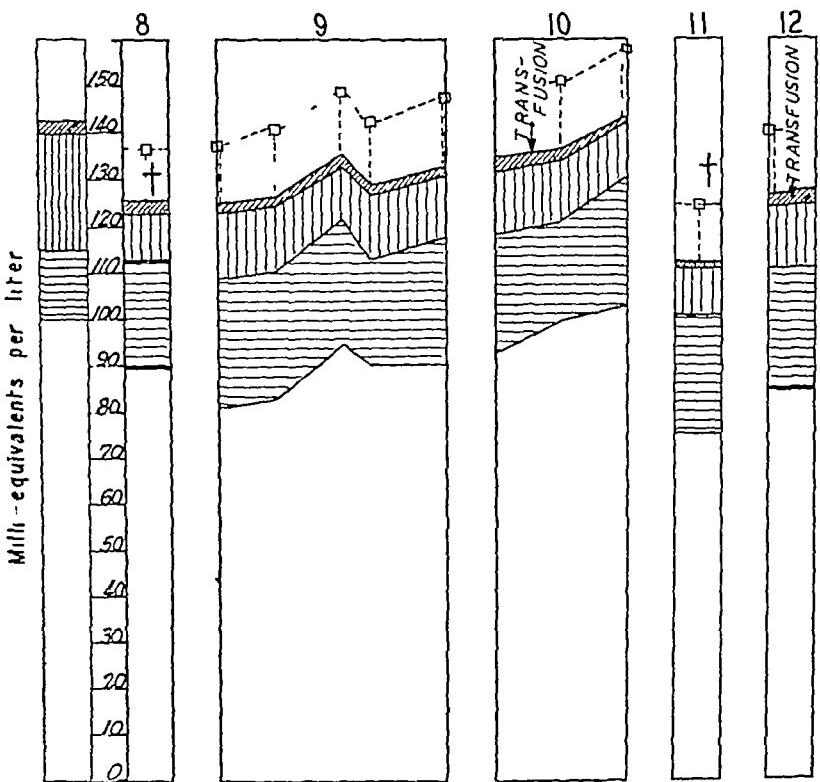
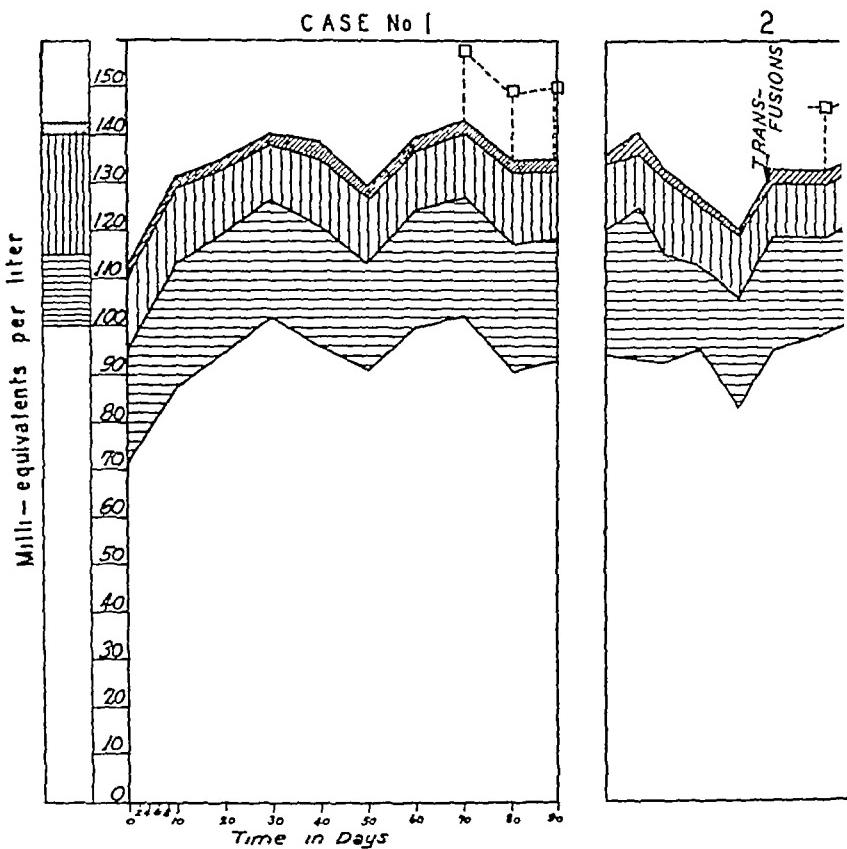


FIG 1A. GRAPHS SHOWING ELECTRO

# BASE

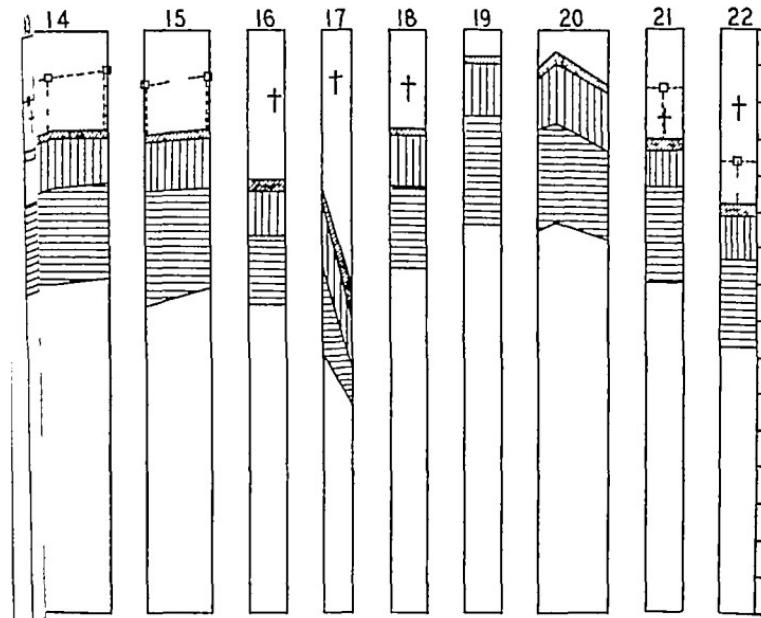
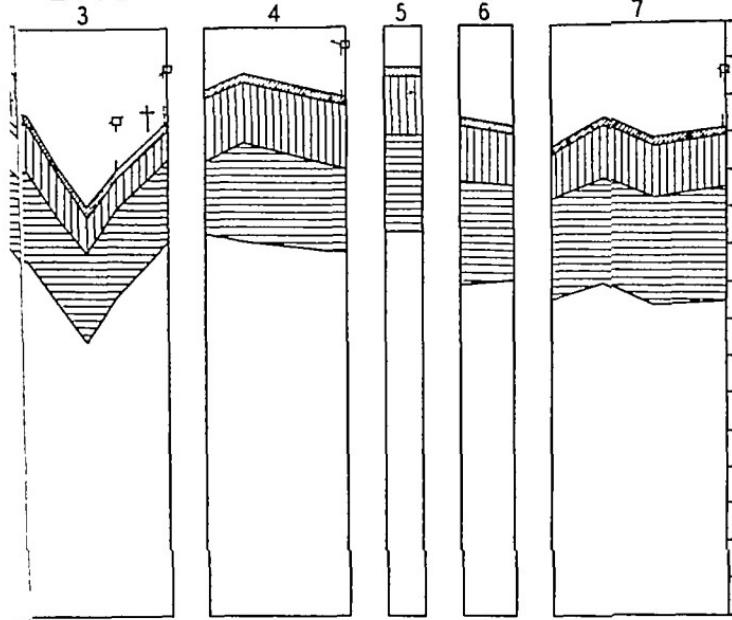


ILLUSTRATION FOR 41 PATIENTS WITH PELLAGRA

mention finding high chloride concentration along with diminished protein concentration in their patients with severe acute pellagra but normal chloride concentrations in the other types of the disease

#### METHODS AND MATERIAL

Venous blood was collected after an over night fast, without stasis and protected from air. The CO<sub>2</sub> combining power of oxalated plasma was measured by the method of Van Slyke (5). Plasma chlorides were determined by the method of Whitehorn (6). For total fixed base of serum the method of Stadie and Ross (7) was used. Methods for determining serum calcium and phosphorus have been given in a preceding article (8).

In determining the acid value of serum proteins use was made of the formula of Van Slyke, Hastings, Hiller, and Sendroy (9). The acid value of phosphorus was obtained by multiplying milligrams of inorganic phosphorus by the factor  $\frac{1.8}{3.1}$  as adopted by Peters, Bulger and Eisenman (10). For calculating the base bound as bicarbonate we used our values for CO<sub>2</sub> combining power instead of CO<sub>2</sub> content. The error thus introduced is probably small.

The patients studied were 42 pellagrins. Detailed clinical and chemical data have been recorded in tabular form in article I of the present series (11). As a rule no attempt was made to supply electrolyte by means other than in the diet previously described (11).

#### RESULTS

The results are presented in tabular form elsewhere (11) and graphically in figures 1a-b, and 2. The outstanding features of the results are also outlined in the summary.

#### COMMENT

Our data would seem to indicate that low serum electrolyte concentration occurs more frequently than does marked disturbance of acid-base equilibrium as indicated by the CO<sub>2</sub> combining power of the plasma. This does not indicate that some of the mechanisms which may produce severe acidosis or alkalosis may not play a major rôle in the disease processes. In recent years leading workers (12, 13, 14) in

this field have stressed the importance of total electrolyte concentration, and Hartmann (15), Marriott and Hartmann (16), Hartmann and Darrow (17), and Hartmann and Elman (18) have brought forth strong evidence to show that abnormal serum osmotic pressure was of greater importance in some of the clinical conditions, which were formerly considered due chiefly to acidosis or alkalosis, than is the actual disturbance of acid base equilibrium. One is justified in suspecting that disturbed serum osmotic pressure may be responsible, in severe pellagra, for the production of generalized cellular injury and death. We are now studying pellagra from this viewpoint.

Our findings for plasma chlorides are at variance with those of Ballif, Rennescu and Reznic (4) who mention finding high chlorides in pellagra.

The tendency toward low concentration of serum electrolytes is most likely due to diarrhea and occasionally to vomiting, both well recognized mechanisms for depleting serum electrolyte. One patient (case 36) who had very low plasma chlorides and serum total base did not show either diarrhea or vomiting during the few days of observation, and, according to the best obtainable history, she had been free of both for the preceding two weeks. There is a slight tendency toward low electrolyte content of the serum in other cases without diarrhea.

The mortality among those patients with greatly lowered concentration of serum electrolytes, and the striking relationship, observed in the other patients, between the clinical severity of the disease and the diminished electrolyte content of the serum, suggest an additional basis from which one may estimate the severity of pellagra, the prognosis, and a rational treatment.

#### ACKNOWLEDGMENTS

Our thanks are due Mr J. H. Arrington for the determinations of total base.

#### SUMMARY

A study of the acid-base equilibrium in pellagra is reported, based on 129 specimens of blood from 42 pellagrins. The data consist of 119 determinations of plasma chlorides, 125 of the CO<sub>2</sub> combining power

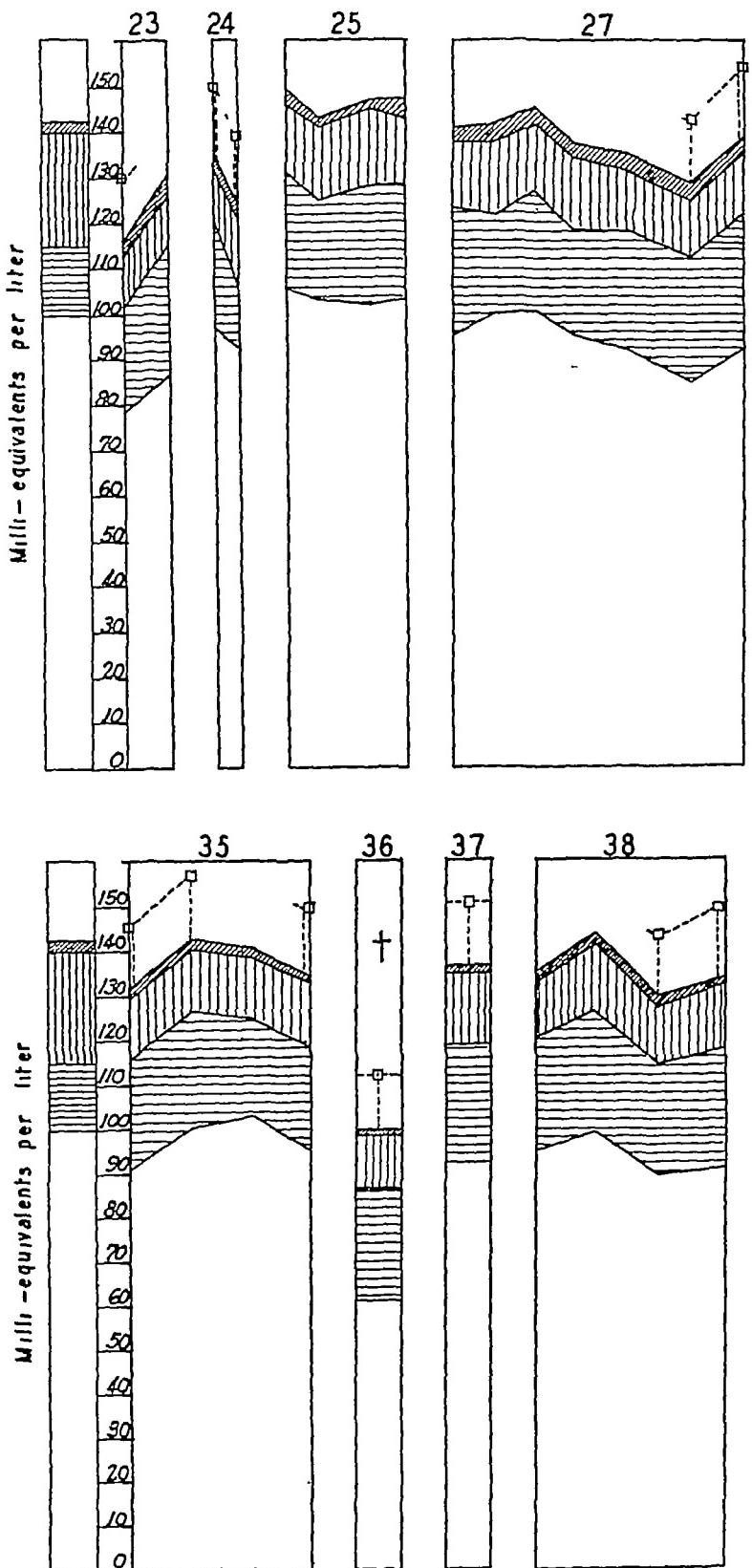
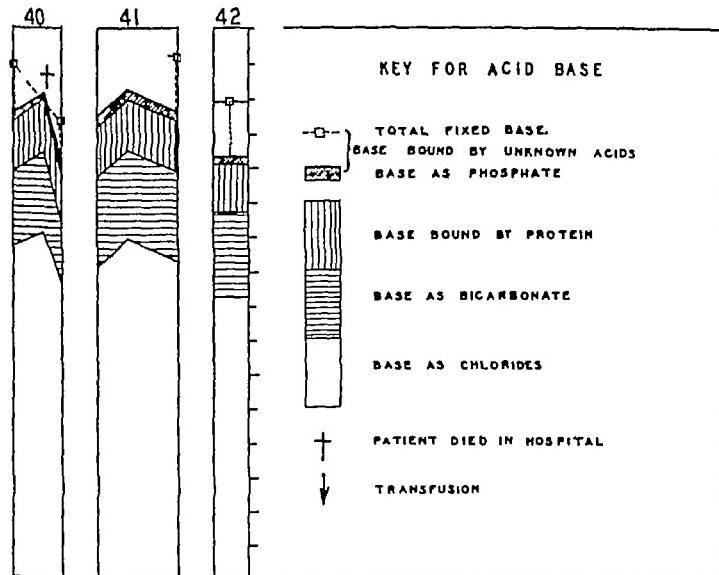
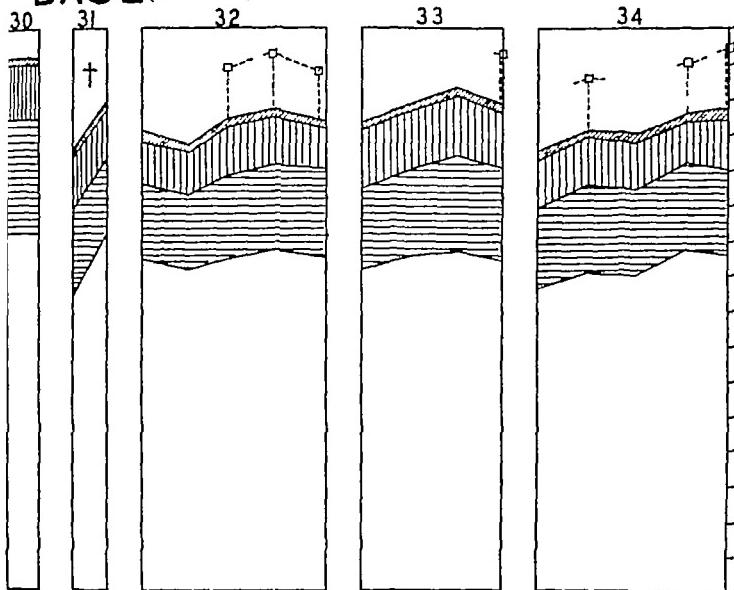


FIG 1B GRAPHS SHOWING I

**BASE (CONTINUED)**



NCENTRATION FOR 41 PATIENTS WITH PELLAGRA

of the plasma, 129 determinations each of serum proteins and inorganic phosphorus, and 53 determinations of total fixed base

The data are presented and analyzed in graphs and histograms

Plasma chlorides tended to be low. The plasma chlorides in 65 per cent of the observations were less than 576 mgm per 100 cc of plasma and in 46 per cent less than 551 mgm per cent, while in 17 per cent they were less than 526 mgm per cent. Two patients showed plasma chlorides of less than 400 mgm per cent.

The maintenance of normal plasma chloride level, by diet alone, even in the absence of vomiting, met with only partial success.

Eighty-four per cent of the determinations for carbon dioxide combining power of the plasma gave results within the normal range, chiefly in the lower portion of the normal range, and 16 per cent were below normal. Marked depletion of plasma bicarbonate was rarely encountered. The observations of Ballif and Gherscovici concerning the carbon dioxide combining power of the blood in pellagra are confirmed.

Marked increase in serum inorganic phosphorus was rarely encountered. Instances seemed to be due to marked oliguria associated with both diarrhea and vomiting.

The sum of the acid values for chloride, carbon dioxide, proteins, and inorganic phosphorus, tended to be low. In 68 per cent of the 118 determinations the sum was less than 136 milliequivalents per liter.

In 32 per cent of the 53 determinations the total fixed base was less than 141, 5 patients showed values of 126 milliequivalents or less. It is probable that the small percentage of low values is misleading, due to the fact that an unduly large proportion of observations were made on convalescent patients.

Four of the five patients in whom the serum total base was less than 126 milliequivalents died soon after study.

The bearing of serum electrolyte concentration upon treatment of pellagra is mentioned.

Diarrhea and occasionally vomiting seem to be chiefly responsible for low electrolyte concentration in the blood serum of pellagrins.

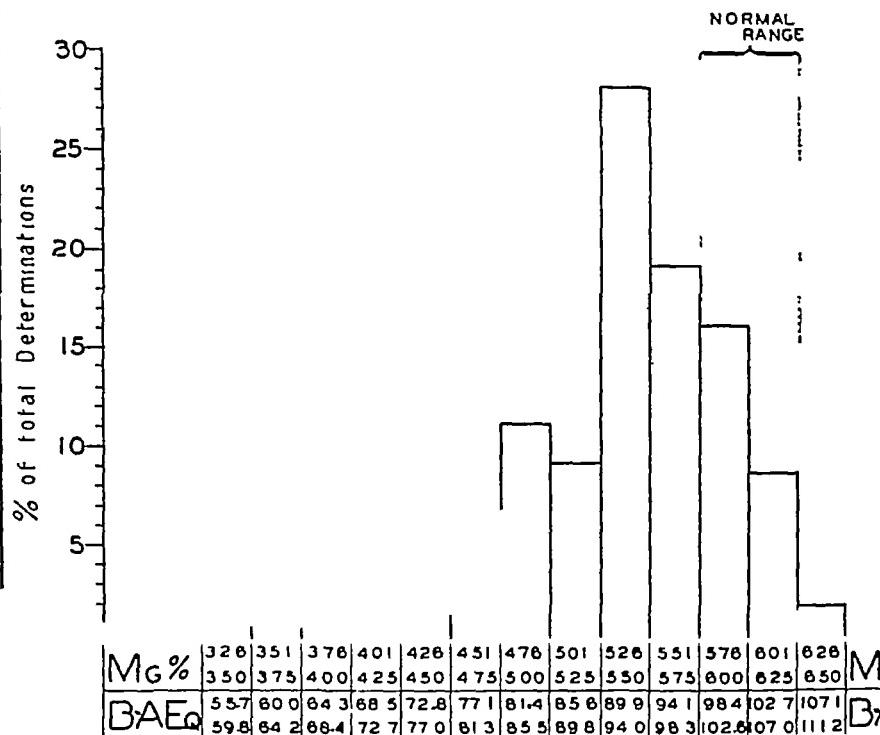
## CONCLUSIONS

Diminution in serum electrolyte concentration appears to be of greater importance in pellagra than disturbances of acid-base equilibrium.

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I



III

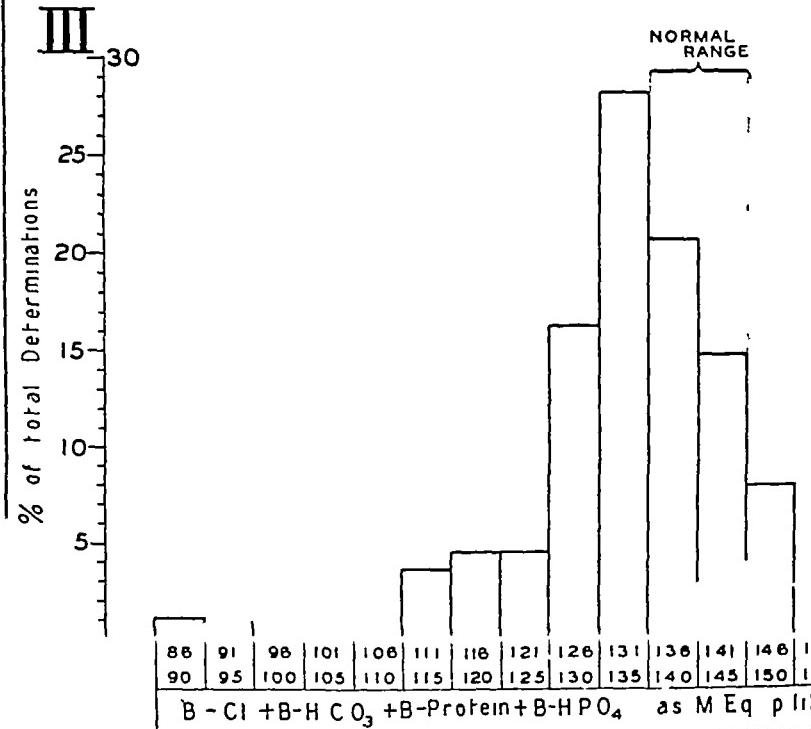
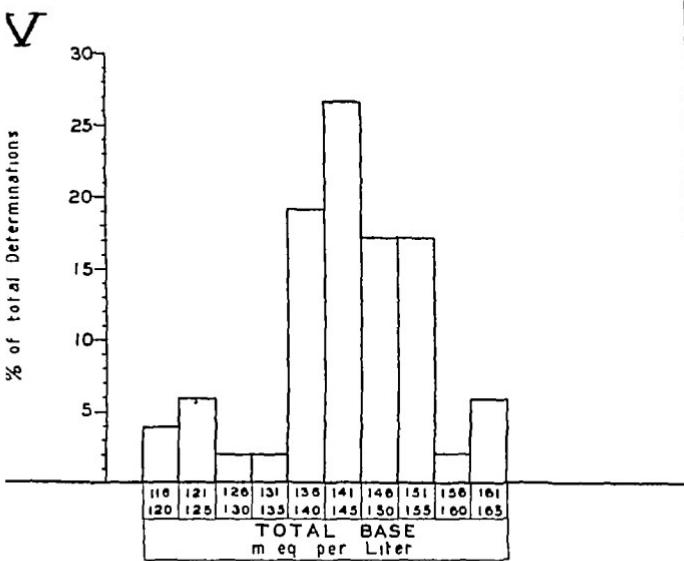
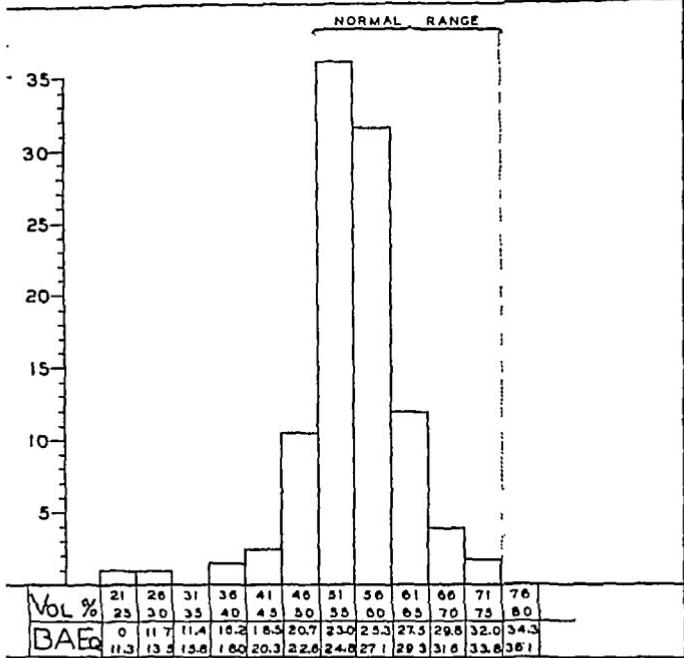


FIG 2 HISTOGRAMS SHOWING DISTRIBUTION

The height of the columns expresses per cent of the total number of determinations on patients during convalescence. I Plasma chlorides. II CO<sub>2</sub> combining power. III Inorganic phosphorus. IV Total base.



#### RUM ELECTROLYTES AND COMBINATIONS THEREOF

fell within that particular range. A large proportion of the observations were made on bicarbonate. III Total acid value for chloride, carbon dioxide, proteins and

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## THE PATHOLOGIC PHYSIOLOGY OF PELLAGRA

### V THE CIRCULATING BLOOD VOLUME

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Determination of circulating blood volume was undertaken as a part of a study of the disturbed physiology in pellagra, chiefly for the purpose of finding out whether shrinkage of plasma volume existed in patients who were suffering from a disease frequently characterized by severe diarrhea. Such a shrinkage would be of great importance of itself, and would obviously have a bearing upon the interpretation of the composition of the plasma determined at the same time. The existence of anemia can hardly be established nor its severity estimated so long as we are in ignorance of the plasma volume. It was also considered possible that the magnitude of the circulating blood volume might be correlated with certain of the features of the skin lesions, such as the degree of exudation of serum.

I have used the dye method of Keith, Rowntree and Geraghty (1) modified as follows. A 3 per cent aqueous solution of brilliant vital red (National Aniline Company) was made up the afternoon before use, and sterilized at 100°C for 8 minutes. With a sterile calibrated pipette the quantity of this solution for each patient was placed in a sterile 50 cc. flask. The volume used for each patient was approximately 1 cc for each 15 kilos of body weight. At the bedside, the dye was drawn from the flask through a sterile needle, which was then discarded, into a sterile Luer syringe which had just been rinsed with sterile distilled water. The dye left in the flask was taken up in a small quantity of sterile distilled water. After taking blood for chemical studies and to provide plasma for preparation of dye standard, the dye was injected through the same needle and the remaining dye washed out of the syringe with the patient's circulating blood by emptying and filling the syringe twice. After removal of the needle

the arm was elevated and no pressure used over the site of the venepuncture. Six minutes after first emptying the syringe of dye, blood was withdrawn from the other arm by venepuncture made a few seconds before. All blood used in the estimation of plasma volume was collected in an oiled syringe and run under oil with 10 mgm of dry powdered potassium oxalate for each 5 cc of blood. The blood was run under the oil from the syringe through a dry glass tube and rubber connection. Blood was run gently but quickly back and forth from centrifuge tube to syringe in order to insure rapid and complete mixing of oxalate with blood. With these precautions hemolysis was a rare occurrence. Blood was collected only after any stasis incident to venepuncture had disappeared. For determination of cell volume the hematocrit tube of Wintrobe (3) was used. The blood which contained dye was thoroughly mixed by drawing it in and out of a wide mouth 1 cc pipette, and then the hematocrit was filled by means of a large calibre capillary pipette. It was centrifuged at 3000 revolutions for 30 minutes. The upper levels for both erythrocytes and leukocytes were read. The use of this hematocrit makes it possible to take the approximate white cell volume into account in calculations. Five per cent was added to hematocrit reading to allow for cell shrinkage. Two cubic centimeter quantities of plasma were used in preparing the standard and unknown dye solutions for the colorimeter. The final volume for each was 6 cc. Readings were made with a Dubosq colorimeter equipped with 5 cc cups.

Since certain clinical manifestations of pellagra suggest increased capillary permeability, it was considered of importance to determine whether the dye might disappear from the blood stream with abnormal rapidity. Fifteen observations were made on patients during the various stages of pellagra, taking an additional dye-containing specimen of blood after intervals of from 4 to 24 minutes. The dye content of these second specimens showed that the maximum rate of disappearance was 4 per cent per 10 minute period and the average rate was one half as great.

How best to express the values for blood volume in this report has not been easy to decide. Most of the patients were greatly underweight. For some of them blood volume per kilo was normal or high, while blood volume per square meter of body surface was below the

recognized standards. It seems clear that neither method of expressing blood volume is satisfactory for such a group, unless corrections are made for abnormal body size. I have used the formula of Rowntree and Brown (2). It provides corrections for the factors of underweight, overweight and extremes of age. These authors have shown that the value for blood volume in either underweight or overweight individuals, though healthy, tends to deviate more from standard values when expressed according to body weight than when expressed according to body surface. In underweight persons the values per kilo of body weight tended to be high, and for those overweight the values were low.

By the use of the formula of Rowntree and Brown I have calculated the ideal total blood volume and have taken 42.4 per cent of it as ideal volume of red blood cells and 51.6 per cent as ideal plasma volume (3). These values have been converted into cc per square meter of body surface and compared with the actual blood, plasma and red cell volumes per square meter.

The results of the determinations for pellagrins are shown graphically in figure 1. Data are shown in table 1 for 21 patients without pellagra, but showing varying degrees of anemia and undernutrition and some of them diarrhea. The outstanding features of the results are also stated in the summary below.

#### COMMENT

One of the mysterious features of pellagrous skin lesions is the difference in mechanism in severe skin damage which determines whether the lesion will take the wet or dry form. Severity of skin injury is not the explanation since some of the instances of most severe skin damage are those where mummification of the skin occurs. The dry form might be suspected of representing low capillary pressure and permeability and the wet form, high capillary pressure or permeability. Conceivably the magnitude of the circulatory blood volume might be an important part of the mechanism involved, high blood volume, possibly high plasma value, would promote exudation of plasma while low volume would oppose it. The work of Rous and Gilding (4) offers a strong basis for this concept. They have shown that when the blood volume of rabbits is greatly diminished by hemor-

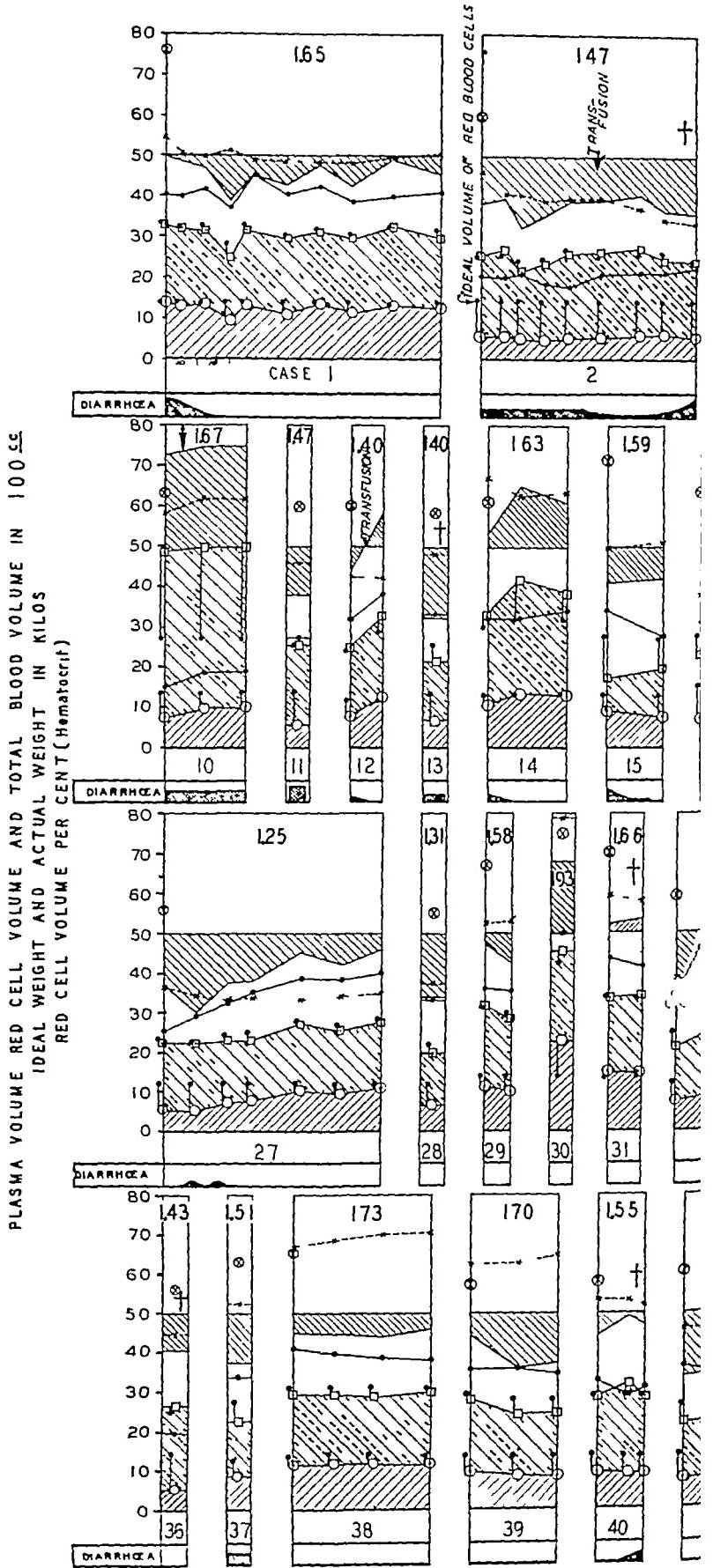
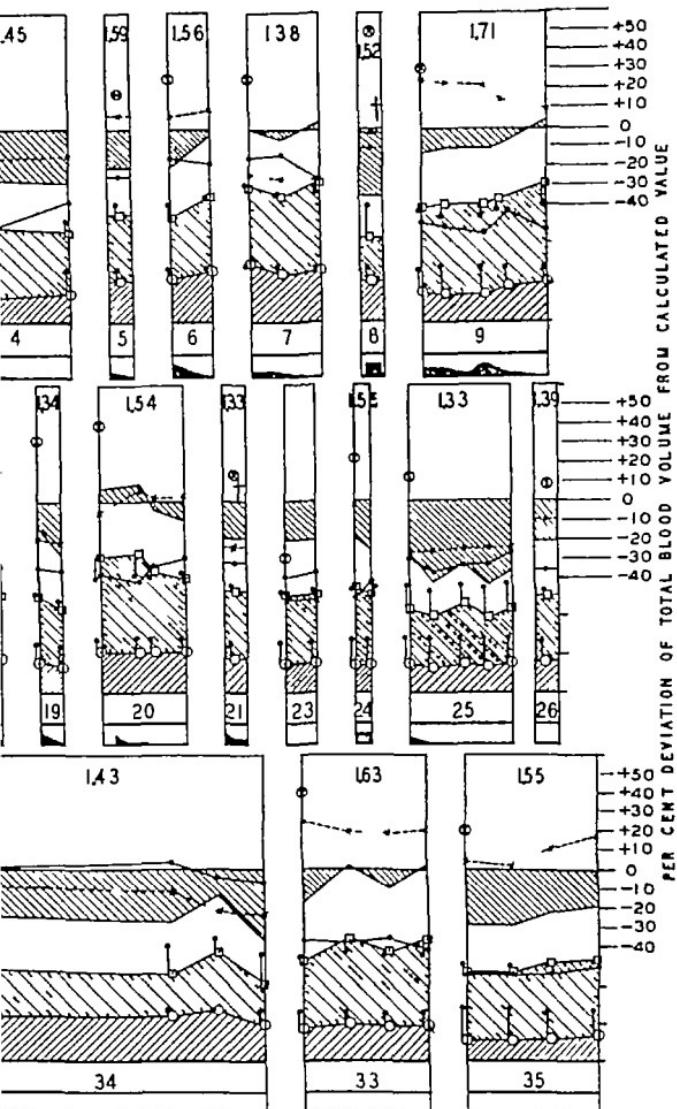


FIG. 1 CIRCULATO

Figures for surface area represent those of initial study Actual and ideal levels of indicated by crosses



### KEY

- { BODY SURFACE IN SQ M
- { IDEAL BODY WEIGHT IN KILOS
- { PER CENT DEVIATION FROM IDEAL  
TOTAL BLOOD VOLUME
- { ACTUAL WEIGHT IN KILOS
- { PER CENT RED BLOOD CELL (HEMATOCRIT)
- { PLASMA VOLUME IN 100% PER SQUARE  
METER OF BODY SURFACE
- { VOLUME OF PACKED RED CELLS IN 100%  
PER SQUARE METER OF BODY SURFACE

↓ TRANSFUSION

DR 41 PELLAGRINS

red cell volume are connected by vertical lines Patients who died in the !

rhage the blood supply to the viscera essential to life is little interfered with, while the skin and skeletal muscles suffer greatly and areas of

TABLE 1  
*Data concerning blood volume of 21 ward patients without pellagra*  
 Standards for normal are same as used for pellagrins

Case number	{ Percentile deviation from normal					Diagnosis
	Plasma volume per cent	Red cell volume per cent	Total blood volume per cent	Weight per cent	Red cell volume (Hemato crit) per cent	
1	+20	-75	-2	-20	-69	Severe pernicious anemia
	+29	-32	+4		-34	Remission
2	+6	-50	-21	-34	-41	Pernicious anemia or sprue Mild diarrhea
3	+4	-27	-22	-20	-67	Severe hookworm anemia
4	+14	+22	+33	+11	-8	Hypertensive heart disease
5	-35	+43	-3	-23	+42	Acute fever Dehydration
	-21	+25	-1	-23	+26	
6	+14	+9	+12	-9	-4	Probably early cancer of stomach
7	+20	-15	+5	-2	-21	Luetic periostitis
8	+14	-18	-0	-29	-21	Cancer of the lung
9	+44	-13	+20	-31	-28	Multiple myeloma
10	-13	+11	-2	-3	+14	Partial obstruction of cecum Constipation No vomiting
11	0	-14	-6	-16	-9	Chronic salpingitis
12	-10	+8	+2	-12	+12	Chronic salpingitis
13	-12	-50	-27	-33	-28	Severe pulmonary tuberculosis
14	+5	-20	-6	-29	-16	Sprue Chronic diarrhea
	+8	-16	-1	-29	-13	
	-9	-26	-16	-29	-14	
15	+9	+12	+10	-26	-1	Mild diarrhea for 30 years
16	+18	-23	+2	-15	-26	Bacillary dysentery
17	-27	+24	-5	+5	+30	Diarrhea of unknown etiology
18	-25	-19	-21	-15	-11	
	-19	-29	-21		-11	Bacillary dysentery
	-17	-29	-27		-11	
19	0	+37	+16	-25	+16	Amebic dysentery
20	-11	+30	+6	0	+19	
	-9	-3	-6		+3	Severe diarrhea of unknown etiology
	-5	+5	+1		+2	
21	-11	-18	-14	-5	-17	Chronic diarrhea

ischemia develop in the skin and remain long after the blood volume has returned to normal. There is good clinical and postmortem evi-

dence to suggest that in chronic pellagra at least the skin may for a long time be deprived of adequate blood supply while actual engorgement of abdominal viscera is present.

Low blood volume may be a factor in the prevention of pellagrous dermatitis in the patients with pellagra sine pellagra. I have observations on a few such patients which are suggestive.

Unfortunately the data here reported offer little evidence bearing upon the part played by the magnitude of blood volume in the determination of the type of skin lesion. Only one patient (case 17) in the group studied showed the mummy-like lesions, which evolved during the period of study. His severe diarrhea and vomiting could not be controlled. Plasma volume showed great diminution at the time of the third study when the injured skin, previously red, had become dry, shrivelled and broken by deep fissures. Later, just before death, the plasma volume returned to its former level, due to administration of large quantities of fluids by hypodermoclysis. It will be noted that the cell volume by hematocrit does not fluctuate inversely as the plasma volume in accordance with the accepted idea. When plasma volume shrinks, one would expect cell volume to rise instead of fall. However, coincident fluctuations in serum proteins (5) are more closely correlated with the estimated plasma volume changes than are the cell volume changes.

The other patients with great variation from normal blood volume had such mild lesions that they could not be classified in either wet or dry group. Only the severe lesions show either marked exudation of serum or immunification. Study of patients before and after development of skin lesions is urgently needed. There is need of a uniformly reproducible experimental skin injury in connection with the study of pellagra. A clinical method for evaluating relative blood service to skin versus abdominal viscera is also needed.

It will be noted that some of the patients with lowest red cell volumes were those who had other diseases besides pellagra. This feature is discussed more fully elsewhere (5).

The blood volume findings for one patient (case 10) are of unusual interest. He had tuberculous peritonitis with fibrous matting of the intestines into large masses and also showed accumulations of ascitic fluid. A prolonged diarrhea probably due to partial intestinal obstruc-

tion preceded the development of pellagra. He had extensive dependent edema and dyspnea on exertion and his heart was enlarged. The blood picture was that of a profound anemia of the microcytic type. His plasma volume was enormous. His circulating red cell volume, though greater than the hematocrit reading would indicate, was still below normal. It seems likely that the circulatory inefficiency was due to the disparity between the great bulk of blood to be circulated, and the low concentration of the oxygen and CO<sub>2</sub> carrying element.

All the data for one patient (case 30) were exceptional. Clinically, he could hardly be called a sick man, the chemical findings were within normal limits, and both plasma and red cell volumes were above normal. He was a robust boiler-maker, though unemployed, he felt fit to work. For many years he had suffered from a gastric neurosis, and vomited following at least one meal each day. However, he had maintained his weight, strength and feeling of well-being almost perfectly. He had omitted lean meat from his diet. Unfortunately he was under observation for only 24 hours. His manifestations of pellagra were almost entirely cutaneous. Study of such exceptional cases should be of the greatest value due to the simplicity of the clinical picture. Observations on this patient are in keeping with the concept previously mentioned in the present series of papers, that many features of the disturbed physiology of pellagra appear to be caused by pellagrous injury to various body systems. These disturbances frequently cause their most definite symptoms after the diagnostic evidences of pellagra have passed.

Many of the patients who showed the lowest volume of circulating red cells were those who had low serum albumin (6) and were greatly underweight. It seems likely that both the low serum albumin concentration and anemia in pellagra may be due to malnutrition. A study of correlation between the two might indicate whether they were due to a common factor. Slight correlation<sup>1</sup> was found to exist between per cent deviation from normal serum albumin, and per cent deviation from ideal volume of red cells. The group of pellagrins studied contained several patients in whom pellagra was complicated

<sup>1</sup>  $r = +0.339 \pm 0.007$

by other diseases. The study of a larger group of patients with uncomplicated pellagra might be more enlightening.

It is well to remember that I have studied patients who were under excellent nursing care. If such studies were made on patients suffering from such neglect as many pellagrins receive in their homes, the results might be quite different.

#### SUMMARY

1 The results of 125 determinations by the dye method of circulating blood volume for 41 pellagrins are presented graphically.

2 Values for plasma volume, red cell volume and total blood volume are compared graphically with standards for normal as determined by the formula of Rowntree and Brown for underweight and overweight individuals.

3 Considering mean values for each patient expressed in terms of per cent deviation from ideal volume the results may be summarized as follows:

*a* For 32 per cent of the group plasma volumes were above normal and for 68 per cent below normal. Plasma volume for 5 per cent of the patients showed a deviation of +21 per cent or greater, 15 per cent showed from +11 per cent to +20 per cent deviation, 12 per cent from +1 per cent to +10 per cent, 42 per cent of the patients showed deviation of from -1 per cent to -10 per cent, 10 per cent from -11 per cent to -20 per cent, 7 per cent showed from -21 per cent to -30 per cent, 7 per cent showed -31 per cent to -40 per cent, and 2 per cent showed deviation of greater than -40 per cent. The median was -4 per cent deviation.

*b* Red cell volumes for 5 per cent of the pellagrins were above normal and for 95 per cent below normal. Ten per cent showed deviation of from -1 per cent to -10 per cent, 12 per cent showed -1 per cent to -20 per cent, 15 per cent showed -21 per cent to -30 per cent, 22 per cent showed from -31 per cent to -40 per cent, 22 per cent showed from -41 per cent to -50 per cent, 15 per cent showed from -51 per cent to -63 per cent deviation from normal standards. The median was -35 per cent deviation.

*c* Total blood volumes for 17 per cent of the pellagrins were above ideal values and 82 per cent were below. Five per cent of the group

showed a deviation from ideal of +31 per cent or greater, 5 per cent showed from +11 per cent to +20 per cent deviation, 7 per cent from +1 per cent to +10 per cent, 17 per cent showed from -1 per cent to -10 per cent, 24 per cent showed from -11 per cent to -20 per cent, 24 per cent showed from -21 per cent to -30 per cent, and 17 per cent of the pellagrins showed a deviation of -31 per cent or greater. The median was -17 per cent deviation from ideal.

4 Data concerning blood volume for 21 patients without pellagra who showed various degrees of anemia, and malnutrition or diarrhea are presented in a table.

5 Anemic patients without pellagra showed plasma volumes higher than normal more frequently than those with pellagra.

6 A possible relationship between the magnitude of blood volume and the clinical features of severe skin lesions is discussed.

7 The plasma volumes of two pellagrins were persistently low for a long time even after the disappearance of any symptom which might explain low plasma volume.

8 There was slight positive correlation between the deviations from normal of serum albumin and of circulating red cell volume.

9 Individual cases of interest are discussed.

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## SKIN REACTIONS OF PATIENTS AND NORMAL INDIVIDUALS TO PROTEIN EXTRACTS OF STREPTOCOCCI<sup>1</sup>

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During the past few years in conjunction with Swift and Hitchcock at the Hospital of the Rockefeller Institute, one of the authors carried out cutaneous tests with nucleoprotein extracts of various streptococci, the results of which have been published (1) (2). Inasmuch as the greater part of that work was done on patients suffering from rheumatic fever it seemed desirable to determine whether a comparable frequency of positive reactions existed in patients with other diseases and in supposedly normal individuals.

Somewhat similar work was done in 1923 by Bristol (3), who performed tests with whole dried streptococci, and found that in 17 individuals with a history of scarlet fever and 31 without there was a positive reaction in 41 and 61 per cent respectively. Both groups are referred to as "fairly normal persons." Mackenzie and Hanger (4) used an alkaline extract of ground up organisms, which they termed "intra cellular antigen," as well as filtrates from plain broth cultures. With these streptococcus derivatives they found a high percentage of positive skin reactions among patients suffering from various diseases but could detect "no relation between the presence or absence of streptococcus allergy and any disease or group of diseases." Some of the strongest reactions observed by them were in presumably normal individuals. Because these authors were unable to demonstrate evidence of free antibodies in the serums of patients either by neutralization tests or by passive transfer in animals, they felt that the allergy or hypersensitiveness represented an acquired reactivity of the cellular tissues as a response to stimulation by bacterial proteins.

Small (5) reported marked general hypersensitiveness in patients

<sup>1</sup> Read by Title before the American Society for Clinical Investigation, May 6, 1929.

with rheumatic fever and arthritis to vaccines and soluble antigens of "Streptococcus cardioarthritidis" injected subcutaneously. This hypersensitive state was manifested by both local and focal reactions. His observations dealt only with the "rheumatic group" of patients.

Birkhaug (6), Kaiser (7), Irvine-Jones (8) and Swift, Wilson and Todd (9) all reported a higher incidence of hypersensitivity among patients suffering from rheumatic infection than among those with other diseases when tested intracutaneously with filtrates of various strains of streptococci. The last named authors suggested that the reaction is caused by an intracellular toxic substance set free when the organisms autolyze in the culture media rather than by a toxin produced and secreted during their growth. Possibly the incitant in these filtrates is, in fact, of the nature of bacterial nucleoprotein.

The studies most comparable with ours are those of Ando and Ozaki (10) (11), who recently have reported skin tests performed on 860 individuals using a nucleoprotein fraction extracted from a strain of *Streptococcus scarlatinae*. These workers made no note of the association of skin hypersensitivity with any particular disease condition but showed that there was a gradual increase in the percentage of positive reactors with the increase in age of the individuals tested (See discussion).

#### METHODS

##### *Cases studied*

During the present study 670 individuals were tested. Of these 475 were definitely diseased patients admitted to the medical wards of the Peter Bent Brigham Hospital. When more than one disease was included in the diagnosis, they were classified according to the primary or most important disorder. Forty-six were at The House of the Good Samaritan all but one of whom were in various stages of convalescence from rheumatic fever or chorea. Ninety-one were either at the Children's or Infants' Hospital, of these 28 were in the general medical wards suffering from various diseases, 39 were infants or premature babies, most of whom presented feeding problems, and 24 had orthopedic conditions but were without a history of recent infection and so were considered as normal controls.<sup>2</sup> In addition 58

<sup>2</sup> We desire to express our thanks to the Staffs of the Children's and Infants' Hospitals and the House of the Good Samaritan for their hearty cooperation.

supposedly normal adults were tested, these included a few patients in whom no evidence of organic disease could be found, members of the resident staff at the Peter Bent Brigham Hospital and students in their final year at the Harvard Medical School

#### *Preparation of the nucleoproteins*

The nucleoproteins used were extracted by Dr Lancefield at the Hospital of the Rockefeller Institute according to the method which has been published (12). They were precipitated repeatedly until their nitrogen content was constant and stored in the form of dried powder. At least once a month a fresh 0.1 per cent solution of each was made for use at the Hospital of the Rockefeller Institute and a portion was sent to us in cold storage. This was kept subsequently in the ice box until used. All solutions were cultured both aerobically and anaerobically on liquid and solid media to test for sterility. Dilutions to the required strength were made in normal salt solution immediately prior to performing the skin tests.

Three different nucleoproteins were used in most instances those from hemolytic streptococcus (S43), green streptococcus (V110A) and ordinary baker's yeast. The latter was used for control purposes. Lancefield (13) has shown that the nucleoprotein fraction of each species of gram positive coccus which she studied, was "immunologically similar within the species." Her study included both the hemolytic and green streptococci. Thus a representative nucleoprotein fraction of these two species of streptococci was used.

Early in the study normal salt solution instead of yeast nucleoprotein was employed for control purposes, but as no positive reactions were encountered, this was discontinued later.

Ten milligrams of these nucleoprotein extracts can be injected intravenously in normal rabbits without eliciting any recognizable reaction. One-tenth milligram when injected intradermally in normal rabbits produces a mild local reaction. This latter dose, however, is many times that which was used in patients reported in this work. We feel therefore that the skin reactions which occurred were not due primarily to any toxic properties of the test materials used.

*Method of making and reading the test*

At the start 0.01 mgm of each nucleoprotein dissolved in 0.1 cc normal salt solution was used as the test dose. Since many of the reactions were unusually marked, it was felt that the results would be more valuable if smaller amounts were injected. Therefore, for most of the work amounts of 0.001 mgm of hemolytic streptococcus nucleoprotein and 0.003 mgm of each of the others were used as test doses.

All tests were done in the skin of the forearm except in a few infants who were tested over the anterior surface of the leg. According to the observations of Alexander (14) the skin response to allergens in patients with hay fever varies in intensity in different parts of the body, but the response in the skin of the leg is quite as large as that on the forearm. Therefore these tests in infants are comparable to those done on the other subjects. The injections were made into and not beneath the skin. Tuberculin syringes graduated in 0.01 cc and with perfectly fitting 26 gauge needles were used to insure accuracy.

Measurements were made at the end of 24 hours in all instances and usually every 24 hours thereafter for at least 3 days with calipers similar to those described by Swift, Wilson and Todd (9). The two greatest diameters at right angles to one another were measured, and the height of all lesions either measured or estimated. After a time one becomes quite accurate in estimating the height of most lesions.

The volume of the lesions was calculated according to the formula used by Derick and Swift (15). Although it is recognized that such results are only approximate, they are, nevertheless, valuable as a basis for comparison. For purposes of presentation (table 1) the volumes of lesions have been transposed to a system of plus values thus, volumes of less than 50 cmm have been considered negative, 51 to 200 cmm as  $\pm$ , 201 to 500 cmm as +, 501 to 800 cmm as ++, 801 to 1100 cmm as +++, and all volumes over 1100 cmm as ++++. In this scheme we have looked upon all  $\pm$  lesions as being doubtful and have classified them as negative. In order to include on a similar basis all patients tested, the volumes of the lesions in the few individuals given the large dose at the start of the work were divided by two. This correction was made, because it was ascertained repeatedly by using both large and small test doses in the same patient.

at the same time that 0.01 mgm of either nucleoprotein extract produced a lesion with a volume twice as great as that which occurred when 0.001 mgm of the hemolytic streptococcus antigen or 0.003 mgm of the green variety was used.

*Description of the reactions*

The type and time of the occurrence of reactions were very similar in all respects to those described by Mackenzie and Hanger (4). A few, occurring early within the space of an hour or two of the time of injection, were of the wheal and erythema type. In practically all instances, however, the reactions reached their maximum intensity after 24 to 30 hours, all readings were made at 24 hour intervals. The

TABLE I  
*Code of arbitrarily selected measurements to show the dimensions and volume of lesions*

Index	Dimensions of lesions mm	Volume of lesions cm mm
		cm mm
0	None to 9 x 13 x 10	Less than 50
+	10.5 x 12.5 x 1.0 to 15.0 x 21.0 x 1.5	51 to 200
+	17.0 x 20.0 x 1.5 to 18.5 x 21.5 x 3.0	201 to 500
++	19.5 x 22.5 x 3.0 to 21.0 x 20.0 x 4.5	501 to 800
+++	19.0 x 23.0 x 4.5 to 25.5 x 34.5 x 3.0	801 to 1100
++++	34.5 x 45.5 x 2.0 or 20.0 x 28.0 x 5.0	Over 1100

response which was elicited, varied markedly, ranging all the way from no reaction or a slight erythema without perceptible induration to a large swelling 6 to 8 cm in diameter and raised 0.5 to 1 cm at the center. These latter consisted of a hard indurated central area, deep red in color, surrounded by an edematous, pinkish red zone which gradually merged with the adjacent normal skin. In a few of the more intense reactions a vesicle containing turbid fluid formed superficially over the center of the lesion. Rarely light red streaks were observed passing from the more intense lesions up the arm. In two instances there was involvement of the regional lymph nodes as manifested by swelling and tenderness. Whenever present these latter manifestations disappeared within a day or so.

Depending upon their severity, the lesions persisted from a day, when mild, to as many as 14 days when there had been marked infiltration.

tion. Usually the skin at the site of reaction underwent a brownish pigmentation and in a few cases desquamated with a fine branny scale. All lesions eventually were absorbed completely, and at no time was there evidence of central necrosis and sloughing. With two or three exceptions no constitutional symptoms were noted.

TABLE 2  
*Results of tests in all individuals grouped into 5 year age periods*

Age	Number of cases tested	Hemolytic streptococcus nucleoprotein (S 43) Intensity of reaction				Green streptococcus nucleoprotein (V 110A) Intensity of reaction			
		Cases		Percentage		Cases		Percentage	
		- or ±	+ to ++++	- or ±	+ to ++++	- or ±	+ to ++++	- or ±	+ to ++++
<i>years</i>									
0-5	61	58	3	95.1	4.9	60	1	98.4	1.6
6-10	49	24	25	49.0	51.0	33	16	67.4	32.6
11-15	40	16	24	40.0	60.0	28	12	70.0	30.0
16-20	31	15	16	48.4	51.6	22	9	71.0	29.0
21-25	63	21	42	33.3	66.7	43	20	68.2	31.8
26-30	49	20	29	40.8	59.2	33	16	67.4	32.6
31-35	52	16	36	30.8	69.2	39	13	75.0	25.0
36-40	35	9	26	25.7	74.3	27	8	77.1	22.9
41-45	53	26	27	49.1	50.9	43	10	81.0	19.0
46-50	60	29	31	48.3	51.7	52	8	86.6	13.4
51-55	55	23	32	41.8	58.2	46	9	83.6	16.4
56-60	51	21	30	41.2	58.8	43	8	84.3	15.7
60 and over	71	39	32	55.0	45.0	62	9	87.3	12.7
Total	670	317	353	47.3	52.7	531	139	79.3	20.7

## RESULTS

As noted previously, 670 individuals in all were tested. In each instance nucleoprotein from hemolytic (S43) and green (V110A) streptococci and a control of either normal salt solution or nucleoprotein from baker's yeast were used.

Table 2 represents an analysis of the 670 individuals tested, arranged in five-year-age periods. This table shows the total number of cases tested in each age group and a division of these into negative or positive reactors. The main points to which attention should be called are

1 That, in the whole group, a much higher proportion (52.7 per cent) reacted positively to the nucleoprotein from the hemolytic streptococcus than to that from the green variety (20.7 per cent)

2 That only very rarely was a reaction observed in an individual under the age of 5 years

3 That in the groups above the age of 5 years, the percentage of positive reactors varied from 45 to 74 per cent with the hemolytic streptococcus nucleoprotein and from 12 to 33 per cent with the green variety. The figures for the patients between the ages of 6 to 10 and 11 to 15 years are misleading inasmuch as nearly half of the children in each of these age groups were suffering from various forms of rheumatic infection, and among these there was an unusually high proportion of positive reactors. Omitting the rheumatic children from these age groups, the percentages of positive reactors are changed from 51 and 60 per cent to 13 and 34.8 per cent respectively for the hemolytic streptococcus antigen, for the green streptococcus, the percentages are lowered from 32.6 and 30 per cent to 0 and 17.4 per cent respectively. The number of patients with rheumatic fever in any one age group above 15 years was insufficient to be of significant influence on the percentage of positive reactors.

When the positive reactors were further analyzed according to the intensity of their response (not shown in the table), it was found that of the 353 who reacted positively to the hemolytic streptococcus nucleoprotein, considerably over half (209) showed a strongly positive (i.e. +++ or +++) response in contrast to only 15 strong reactors among the 139 who reacted positively to the green streptococcus antigen.

In a detailed analysis of the cases under 15 years of age (150 in number) we have observed several interesting facts. As shown in table 3 these individuals have been divided into two groups, non-rheumatic and rheumatic. The non-rheumatic group is comprised of 106 individuals. The rheumatic group contains 44 patients all of whom were in some stage of convalescence from rheumatic fever or chorea. Observing first the reactions to the hemolytic streptococcus nucleoprotein we find that out of the 60 non rheumatic children under the age of 5 years, 58 or 96.6 per cent failed to show any reaction to this antigen. Thus, only 2 positive reactions were observed in the 60 indi-

viduals tested, while in the rheumatic group the one patient tested showed a positive reaction. This one case, of course, has little significance, and due to the infrequency of rheumatic fever at this age, it will be difficult to obtain further data on this point. The correlation between the occurrence of positive skin reactions and rheumatic infection is brought out strikingly in the children over 5 years of age. In the 6 to 10 year age group, of the 23 non-rheumatic children, only

TABLE 3  
*Results of tests in young individuals*

Age	Number of cases tested	Hemolytic streptococcus nucleoprotein (S 43) Intensity of reaction						Green streptococcus nucleoprotein (V 110A) Intensity of reaction								
		Cases			Percentage			Cases			Percentage					
		- or ±	++	+++ or ++++	- or ±	++	+++	- or ±	++	+++ or ++++	- or ±	++	+++			
<i>years</i>																
Non-rheumatic	0-5	60	58	2	0	96	6	3	4	60	0	0	100	0	0	
	6-10	23	20	3	0	87	0	13	0	23	0	0	100	0	0	
	11-15	23	15	4	4	65	2	34	8	19	2	2	82	6	17	4
	Total	106	93	9	4	87	7	12	3	102	2	2	96	2	3	8
Rheumatic	0-5	1	0	1	0	*	*	0	1	0	*	*	*	*	*	
	6-10	26	4	11	11	15	4	84	6	10	14	2	38	4	61	6
	11-15	17	1	3	13	5	9	94	1	9	8	0	53	0	47	0
	Total	44	5	15	24	11	4	88	6	19	23	2	43	2	56	8

\* Too few to treat statistically

3 or 13 per cent reacted positively while in the 26 rheumatic children 22 or 84.6 per cent showed a positive reaction. In the 11 to 15 year age group, there were 8 positive reactors out of 23 (34.8 per cent) in the non-rheumatic children compared to 16 out of 17 (94.1 per cent) in the rheumatic ones. It is noteworthy that among the rheumatic children who had a positive skin reaction, well over half (24 out of 39) were strongly positive (+++ or ++++), whereas there were but 4 strong reactors out of the 13 non-rheumatic children who showed a positive reaction.

An even more striking preponderance of positive tests is seen in an analysis of the results with the green streptococcus antigen. Here none of the non rheumatic children under 10 years showed anything more than a doubtful reaction and but 17.4 per cent of those from 11 to 15 were positive whereas of the 44 rheumatic children 25 or 56.8 per cent reacted positively.

These figures reveal that the "peak" of positive skin reactors among children tested with our streptococcal antigens is considerably influenced by the presence or absence of rheumatic infection. Omitting the rheumatic individuals from the different age groups, as noted above, we find a gradual rise in the percentage of positive reactors as the children grow older in these first three age groups from 3.4 per cent to 13 per cent to 34.8 per cent with the hemolytic streptococcus antigen and from 0 to 0 to 17.4 per cent with the green variety. With the rheumatic children the percentages are much higher in each group. This finding of a high incidence of skin hypersensitivity in patients suffering from rheumatic fever is in keeping with the observations of Swift and his co workers and others who have used various streptococcus products in their studies, and it emphasizes the important association of such hypersensitivity with rheumatic infection.

In table 4 data from all the patients, irrespective of age, have been analyzed according to the chief disease from which each suffered. In addition there are three other groups, (no 10) 82 so-called normal individuals, (no 21) 28 children, and (no 22) 39 infants and premature babies. The two latter groups were not sufficiently large to analyze according to disease. The table indicates the number of individuals tested, the intensity of reaction and the proportion of positive and negative reactors in each disease group. The size of the divisions varies considerably, but all are large enough to be of value. The order of arrangement is according to the percentage of positive reactions to the hemolytic streptococcus nucleoprotein. Here, as pointed out from table 2, one finds that there is a much higher proportion of positive reactors to this nucleoprotein than to that from the green streptococcus. This is true for the individual disease groups as well as for the entire series.

A few points respecting several of the divisions of this table are worthy of special mention. In the first place all patients suffering

TABLE 4  
Results of tests in all individuals grouped according to disease

Disease group	Number of cases tested	Hemolytic streptococcus nucleoprotein (S 43)										Green streptococcus nucleoprotein (V 110 A)										
		Cases					Percentage					Cases					Percentage					
		-	+	++	+++	++++	-	+	++	+++	++++	-	+	++	+++	++++	-	+	++	+++	++++	
1 Rheumatic fever or chorea	59	3	4	10	11	2	29	11	9	88	1	14	15	20	8	2	0	49	2	50	8	
2 Liver or gallbladder disease	8	1	0	4	0	0	3	12	5	87	5	3	3	1	1	0	0	0	75	0	25	0
3 Peptic ulcer	57	7	7	7	4	6	26	24	6	75	4	25	16	8	5	2	1	1	71	9	28	1
4 Tonsillitis or sinusitis	12	3	0	2	0	2	5	25	0	75	0	7	1	0	2	1	1	1	66	6	33	4
5 Diabetes	20	3	2	5	4	1	5	25	0	75	0	12	2	5	1	0	0	0	70	0	30	0
6 Hyperthyroidism or myxedema	12	1	2	1	0	2	6	25	0	75	0	5	5	2	0	0	0	0	83	3	16	7
7 Arthritis	39	3	8	5	6	6	11	28	2	71	8	23	8	4	1	0	3	79	5	20	5	
8 Hypertension	21	5	3	4	0	1	8	38	1	61	9	14	2	4	1	0	0	76	2	23	8	
9 Miscellaneous group	54	15	8	5	5	6	15	42	6	57	4	34	8	7	4	1	0	77	8	22	2	
10 Normal group	82	25	12	9	3	6	27	45	1	54	9	50	11	20	1	0	0	74	4	25	6	
11 Chronic myocarditis	34	12	5	4	6	0	7	50	0	50	0	23	8	2	1	0	0	91	1	8	9	
12 Nephritis	30	10	5	3	2	2	8	50	0	50	0	17	3	7	1	1	1	66	6	33	4	
13 Asthma and chronic bronchitis	23	6	6	2	3	2	4	52	1	47	9	17	3	3	0	0	0	87	0	13	0	
14 Influenza	34	11	8	11	2	0	2	55	8	44	2	24	4	5	1	0	0	82	3	17	7	
15 Chronic cardiac valvular disease	29	10	8	6	2	1	2	62	1	37	9	21	3	5	0	0	0	82	7	17	3	
16 Syphilis	15	7	3	1	1	0	3	66	6	33	4	12	0	1	0	0	2	80	0	20	0	
17 Carcinoma	23	11	4	2	1	1	4	65	2	34	8	20	3	0	0	0	0	100	0	0	0	
18 Pneumonia	30	19	3	4	1	1	2	73	4	26	6	24	6	0	0	0	0	100	0	0	0	
19 Subacute bacterial endocarditis	4	3	0	1	0	0	0	75	0	25	0	4	0	0	0	0	0	100	0	0	0	
20 Tuberculosis	17	9	4	2	0	0	2	76	5	23	5	11	4	2	0	0	0	88	2	11	8	
21 Children (under 12 miscellaneous)	28	15	7	4	1	0	1	78	6	21	4	25	2	0	1	0	0	96	4	3	6	
22 Infants and premature babies	39	34	5	0	0	0	0	100	0	0	0	34	5	0	0	0	0	100	0	0	0	
Total	670	213	104	92	52	39	170	47	3	52	7	419	112	96	28	7	8	79	3	20	7	

from rheumatic fever or chorea irrespective of age were more frequently hypersensitive to the antigens of both hemolytic and green streptococci than were those in any other disease group. Of the 52 patients in this division, who showed a positive reaction to the hemolytic streptococcus nucleoprotein, 29 or 55.8 per cent showed a ++++ response. Secondly, whereas the percentage of positive reactors to this antigen (88.1 per cent) is only slightly higher than that shown in several other disease groups, the percentage of those reacting positively to the green streptococcus nucleoprotein (50.8 per cent) is with four exceptions more than double that shown by any other disease group. These points emphasize, as noted above, the strong tendency in individuals suffering from rheumatic infection to show a high degree of skin hypersensitivity to these antigens. In contrast to this, of the 29 patients suffering from chronic cardiac valvular disease, only 11 or 37.9 per cent gave a positive reaction to the hemolytic streptococcus antigen and only 3 or 10.3 per cent a strongly positive response. All of these 29 patients had had rheumatic heart disease, but there was no evidence of recent active rheumatic infection, indicating that the high incidence of hypersensitivity seen in the early course of rheumatic disease does not persist in later years.

Of the four patients with subacute bacterial endocarditis due to *Streptococcus viridans*, only one showed a mildly positive test to the hemolytic streptococcus nucleoprotein and none to the green variety. This is a very small number of patients from which no deduction can be drawn. The finding, however, is in keeping with the observations of Kinsella and Garcia (16), Howell and Corrigan (17) and Swift (18), who have noted a failure of patients suffering from this disease to show skin reactions when tested with living streptococci or their derivatives.

Of considerable interest to us was the high incidence of positive reactors to the hemolytic streptococcus nucleoprotein among the patients with gastric or duodenal ulcer. Three fourths of these patients showed a positive reaction and of these (43 in number) 26 or 60.4 per cent were very strongly positive. It is impossible to draw any definite conclusion from this fact any more than is possible from the similar high incidence in the rheumatic fever group, however, it may be looked on as evidence favoring the conception held by some that peptic ulcer is associated with streptococcus infection. It is

worth noting that the two patients with ulcer reported by Mackenzie and Hanger (4) gave strong reactions to streptococcal antigen

The proportion and degree of hypersensitivity shown in the diabetic and thyroid groups were rather unexpected, and no explanation for these findings is offered. The arthritis group included all types of arthritis other than that of rheumatic fever. Various stages of chronicity of the different forms were encountered. There was no very evident correlation between the types of arthritis thought to be streptococcal in origin and their skin hypersensitivity to the nucleoproteins.

TABLE 5  
*Results of tests in patients suffering from various forms of nephritis*

Type of nephritis	Number of cases tested	Reactions to hemolytic streptococcus nucleoprotein (S 43)—cases			Reactions to green streptococcus nucleoprotein (V 110A)—cases		
		— or ±	+ or ++	+++ or ++++	— or ±	+ or ++	+++ or ++++
Acute	1	0	1	0	1	0	0
Subacute hemorrhagic	10	3	2	5	4	4	2
Chronic without edema	12	8	1	3	10	2	0
Nephrosis	7	4	1	2	5	2	0
Total	30	15	5	10	20	8	2

The miscellaneous group included various diseases such as epilepsy, lead poisoning, mucous colitis and others too few in number to be discussed separately. Our finding that slightly over half of the so-called "normals" were hypersensitive to the hemolytic streptococcus nucleoprotein was not unexpected, since many gave a history of previous attacks of tonsillitis or other infection commonly attributed to the streptococcus. The low incidence of positive reactions in the disease groups known to be due to unrelated microorganisms—tuberculosis, syphilis and pneumonia—again was not unexpected.

Taken as a group, the children, excepting those with rheumatic infection, gave the lowest percentage of positive reactions to the hemolytic streptococcus nucleoprotein (21.4 per cent), only 3.6 per cent gave any positive response to the green variety. There were no

positive reactions to either antigen among the infants and premature babies

One disease group worthy of special attention is that with the diagnosis of nephritis. This included 30 patients of whom 50 per cent reacted positively to the hemolytic streptococcus nucleoprotein and 33 4 per cent to the green variety. A subdivision of these patients according to the type of nephritis from which they suffered (table 5) shows that not only did more of them react to the hemolytic than to the green streptococcus antigen, but also the intensity of this reaction was definitely more marked. Again, patients with acute or subacute nephritis showed a much higher incidence of reactions than did the chronic cases without edema or those with so called nephrosis.

In no instance did any of the individuals tested with the nucleoprotein from baker's yeast show what might be considered a positive reaction.

#### DISCUSSION

The purpose of this study was to ascertain the type and degree of response to an intradermal injection of streptococcus antigens in a large group of general hospital patients. Much work has been done by Swift and his collaborators (1) (2) and by Birkhaug (6), Kaiser (7), Irvine Jones (8) and others in carrying out similar tests on patients suffering from the "rheumatic series" of diseases. Few of these workers have reported results in any large group of individuals with other diseases than rheumatic fever, though in many instances the control cases have been hospital patients with a variety of ailments. Our studies carried out on 670 hospital patients and normal controls showed that positive results may be obtained in a great variety of disease conditions so that the test has no specific diagnostic value. These results probably are not entirely comparable to those of other workers, since most of them have used as test materials either filtrates of cultures or unpurified extracts of the various streptococci. In this study the nucleoprotein fraction of two types of streptococci was used. This fraction in each instance had been reprecipitated until its nitrogen content was constant, then diluted and treated so that the potency of the test antigen presumably was constant throughout. It should be noted that, although the two streptococcus nucleoproteins used con-

tained approximately the same amount of nitrogen (12.9 per cent), they varied markedly in their ability to elicit reactions. This variability closely paralleled the known virulence of the two species of streptococci from which the nucleoproteins were obtained. Thus, in nearly every instance was the response to the hemolytic streptococcus antigen (S43) greater than that to the green variety (V110A). This is even more striking in view of the fact that the dose of the first was only one-third as large as that of the second.

No satisfactory explanation for this observation is available. Since both antigens were prepared in the same manner, it does not seem likely that one was denatured more than the other during preparation. Whether this is an indication of a higher degree of sensitivity of the tissues to the hemolytic streptococcus antigen, of the greater prevalence of this type of streptococcus as a pathogenic organism, or of some unknown element of greater toxicity in this antigen as compared to the other cannot be said.

Does the age of the individual tested play any part in determining tissue sensitivity to this nucleoprotein fraction of streptococci? If those patients suffering from rheumatic fever, of which there was a large number in the 6 to 10 and 11 to 15 year age groups are eliminated, then it can be stated that under the age of 5 years very few are sensitive, that between the ages of 6 and 15 years there is an increase in frequency of sensitiveness, and above 15 years there is no indication that age plays any significant rôle. These findings are quite in accord with those reported previously by Mackenzie and Hanger (4), who noted a failure on the part of young children to show skin hypersensitivity to streptococcus products, and to those of Ando and Ozaki (11), who found that only 14.6 per cent of children between the ages of 2 and 5 years showed a positive reaction to streptococcus nucleoprotein, whereas above the age of 15 years 67.8 per cent of those tested reacted positively. All the strong (+++) reactions which these workers observed, were in individuals over 10 years of age. The fact that nearly all of the young patients tested by us failed to give a positive response would suggest that none of them had harbored infections due to streptococci of sufficiently long duration or intensity to allow their tissues to become sensitized to these organisms. Furthermore, this finding that nearly all of the children under 5 years of

age failed to react, whereas between the ages of 15 and 20 years somewhat over half of the individuals tested showed some degree of hypersensitiveness is comparable to the observation of Opie, Landis, McPhedran and Hetherington in their work on tuberculosis (19) These authors reported that in families not harboring a source of contagion only 20 per cent of the children at the age of 5 years showed a positive tuberculin reaction, whereas about 100 per cent in similar families at the age of 20 years gave a positive test.

The question arises as to what relation the occurrence of a positive skin reaction, considered as evidence of hypersensitiveness, bears to disease in any individual. It is impossible to answer this definitely at the present time. The figures in table 4 show that this hypersensitive state is encountered more frequently in some diseases than in others. For instance, the patients suffering from rheumatic fever gave an incidence of 88.1 per cent positive tests as compared to 54.9 per cent in the group of so-called normal individuals tested with the hemolytic streptococcus nucleoprotein. We feel that this high incidence of positive reactors may well be of some significance, though one can go no further than state that it suggests the possible rôle of the streptococcus as an etiological factor in rheumatic infection. There is further interest in the observation that individuals suffering from diseases not infrequently attributed to streptococcal infection such as tonsillitis, sinusitis, arthritis, and acute or subacute nephritis gave a much higher proportion of positive skin reactions to the hemolytic streptococcus antigen than did individuals with diseases known to be due to quite unrelated organisms, namely syphilis, tuberculosis and pneumonia. There are also several disease groups (peptic ulcer, diabetes and thyroid disease), in which about three fourths of the patients were hypersensitive, although the causative factors of these disturbances are unknown, and their relationship to streptococcal infection although suggested has never been proved. The occurrence of a high proportion of positive reactors among patients with acute or subacute nephritis is in keeping with the recently published findings of Hansen Pruss, Longcope and O'Brien (20). These authors found a high incidence and degree of hypersensitiveness to filtrates of various strains of streptococci among patients suffering from these forms of nephritis. Although the test antigens used in their observations and

ours were not prepared in the same manner, it is possible that theirs contained a considerable amount of nucleoprotein from autolysis during growth or that ours contained a toxic substance. If this is true, then the similar findings have a common significance, namely, a hypersensitive state of any or all the tissues of the body to streptococcus derivatives.

Is this hypersensitive state necessarily beneficial to the individual and one to be desired? It is probable that, could an efficient immune state be obtained without concomitant hypersensitivity, less injury would be inflicted on the tissues at time of reinfection. There is evidence that it is harmful to the living body. As shown by Derick and Swift (15), when rabbits, made hypersensitive by repeated intracutaneous injections of live streptococci, are injected subsequently either into the tissues or intravenously with minimal doses of the same or similar organisms they show respectively either localized fulminating lesions or acute widespread reactions often terminating in death. Control animals, tested in like fashion, show little or no reaction.

Rich and McCordock (21) in their work on tuberculosis believe that hypersensitivity to tubercle protein may not be necessary for the production of immunity and suggest that probably it would be beneficial to desensitize the body if possible "and so free the tissues from the danger of being destroyed by traces of bacillary products." If such be true, it is not sufficient, as has been suggested previously (2) (22), merely to eradicate foci of infection, when these are found in individuals suffering from such diseases as rheumatic fever, acute nephritis and so forth, but an attempt should be made, if such individuals are found to be hypersensitive, to carry them by suitable vaccination from this to an immune state in which they can respond to a maximum insult with a minimum of tissue reaction. Such work, started in patients with rheumatic fever (23), appears to favor such a conception, but time will determine its ultimate value.

#### SUMMARY

1 Intracutaneous tests, made with the purified nucleoprotein fraction of a hemolytic and green streptococcus were studied in a group of 670 individuals, most of them patients in the medical wards of a

general hospital. The antigens used were purified till their nitrogen content was constant. A similar protein from baker's yeast was used as a control in part of the work.

2 The hemolytic streptococcus nucleoprotein almost uniformly induced stronger reactions than did the green streptococcus antigen.

3. In individuals over 15 years, age plays no part in determining the presence or absence of sensitiveness to these antigens. In children under 15 years of age there is with increase in age a gradually increasing proportion of positive to negative reactors. This proportion is influenced markedly by the presence or absence in children of rheumatic infection, the percentage of positive reactors among the rheumatic group outnumbering that in the non-rheumatic group by a wide margin.

4 Since positive reactions may be obtained in a great variety of diseases, the test has no specific diagnostic value.

5 The disease from which one suffers at the time of testing appears to bear some relationship to the state of sensitiveness. Whether this is cause and effect or merely an association more apparent than real, the present work does not reveal.

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## STUDIES IN CONGESTIVE HEART FAILURE

### IX THE EFFECT OF DIGITALIS ON THE POTASSIUM CONTENT OF THE CARDIAC MUSCLE OF DOGS

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Previous investigations (1, 2) have shown that the potassium content of the cardiac muscle is decreased in patients dying of congestive heart failure. This diminution was found only in dilated hearts and appeared to be related to overwork and the attendant fatigue. As digitalis is the sovereign remedy in the treatment of cardiac fatigue it was thought that knowledge of its effect on the potassium content of the heart was desirable.

The drug was given subcutaneously as Digifolin (Ciba) to dogs in doses of varying size. The plan of administration consisted in giving the equivalent of a full therapeutic dose in man, i.e., 0.2 to 0.3 cc. per kilogram of body weight in twenty-four to forty-eight hours and then giving larger or smaller daily "maintenance" doses, according to whether "toxic" or "therapeutic" effects were desired. At intervals varying from one to thirty seven days after the administration of the drug had been started the animals were killed, and their hearts were analyzed for potassium according to the technique which has been described in our previous studies. Daily pulse counts and electrocardiograms were made in some of the animals.

Twenty-seven animals were used in the course of the experiment and they were divided into four groups on the basis of the amount of digifolin given them. The "control" group which numbered ten, received no digitalis, three of these were healthy normal dogs which were killed by bleeding, the other seven animals had been utilized for various types of experimental studies which did not include the administration of digitalis. The second group comprised ten animals to whom digifolin was given in "therapeutic" doses—by which is

meant in amounts insufficient to produce toxic symptoms. The most characteristic toxic symptom in dogs being vomiting, no animal who vomited or who appeared very ill whether or not there was actual vomiting, was put in this group. Seven animals received digifolin in "toxic" doses, four of these were killed and three given sufficient digifolin to cause death (i.e., "fatal" doses). It will be noted that some of the animals in the toxic group received less of the drug than did many in the "therapeutic" group, but they usually received more

TABLE 1

*The solids and potassium content of the cardiac muscle of dogs not receiving digitalis*

Animal number	Right ventricle			Left ventricle			Remarks
	Solids <i>per cent</i>	Potassium in dry tissue <i>per cent</i>	Potassium in wet tissue <i>per cent</i>	Solids <i>per cent</i>	Potassium in dry tissue <i>per cent</i>	Potassium in wet tissue <i>per cent</i>	
N <sub>1</sub>	22.9	1.50	0.345	23.0	1.33	0.314	Normal
N	21.5	1.76	0.378	22.3	1.48	0.330	Normal
N <sub>2</sub>	22.8	1.51	0.365	22.8	1.40	0.320	Normal
E B 22	20.3	1.50	0.305	21.4	1.70	0.364	Barbital spinocaine
E B 23	20.7	1.29	0.266	20.9	1.30	0.272	Barbital spinocaine
E B 24	21.0	1.86	0.390	20.8	1.65	0.342	Barbital spinocaine
E B 25	21.8	1.44	0.313	22.0	1.39	0.306	Barbital spinocaine
L B 26	21.4	1.60	0.343	22.0	1.45	0.320	Barbital spinocaine
A B 2				22.4	1.37	0.307	Traumatic shock
A B 3				21.9	1.35	0.296	Traumatic shock

in proportion to the length of time the drug was administered. Vomiting was considered the criterion of toxicity. The three animals in the "fatal" group received large amounts (4.8 to 11.0 cc per kilo) of digifolin and death was due to the action of the drug.

#### RESULTS

The changes in the clinical states, pulse rates and electrocardiograms were the same as are usually found in dogs to which digitalis has been given. These have been considered in detail by other authors and need not be discussed here.

The values for the solids and the potassium content of the hearts

TABLE 2

*The total solids and potassium content of the cardiac muscle of dogs receiving digitalis*

Animal number	Duration of experiment days	Total amount digitalis given cc per kilo	Right ventricle			Left ventricle			Vomiting	Remarks
			Solids	Potassium in dry tissue per cent	Potassium in wet tissue per cent	Solids	Potassium in dry tissue per cent	Potassium in wet tissue per cent		
D <sub>7</sub>	1	0.25	1.24	21.8	1.28	0.280	0	Killed		
D <sub>8</sub>	1	0.25	20.9	1.34	0.280	21.3	1.40	0.298	0	Killed
D <sub>9</sub>	2	0.60	21.7	1.71	0.370	22.0	1.45	0.319	0	Killed
D <sub>10</sub>	2	0.60	20.9	1.69	0.353	20.2	1.73	0.349	0	Killed
D <sub>11</sub>	1	0.55	22.1	1.06	0.234	23.0	1.46	0.337	0	Killed
D <sub>12</sub>	4	1.4	22.0	1.17	0.258	22.6	1.45	0.327	0	Killed
D <sub>13</sub>	4	1.4	22.6	1.29	0.290	23.0	1.49	0.342	0	Killed
D <sub>17</sub>	30	1.31	18.1	1.21	0.218	18.7	1.00	0.187	0	Killed
D <sub>18</sub>	37	1.52	20.3	1.56	0.317	21.2	1.42	0.301	0	Killed
D <sub>19</sub>	36	1.57	20.9	1.55	0.324	22.4	1.30	0.291	0	Killed
D <sub>12</sub>	7	0.75	22.0	0.99	0.217	21.3	1.14	0.243	++	Killed
D <sub>13</sub>	14	0.95	20.1	1.15	0.231	20.3	1.09	0.221	+	Killed
D <sub>14</sub>	21	1.10	20.9	0.99	0.208	21.9	1.00	0.219	+	Killed
D <sub>4</sub>	11	5.0	20.8	1.16	0.240	21.1	0.90	0.190	+++	Killed
D <sub>1</sub>	8	4.8	20.2	0.85	0.172	22.2	1.02	0.228	+++	Died
D <sub>2</sub>	8	5.7	20.6	0.98	0.201	20.5	1.07	0.217	+++	Died
D <sub>3</sub>	17	11.0	27.5	0.83	0.227	21.2	1.10	0.243	+++	Died

TABLE 3

*Average values for the solids and potassium content of the cardiac muscle of dogs, arranged according to the amount of digitalis administered*

Amount of digitalis received	Number of animals	Right ventricle			Left ventricle		
		Solids	Potassium in dry tissue per cent	Potassium in wet tissue per cent	Solids	Potassium in dry tissue per cent	Potassium in wet tissue per cent
None	10	21.5	1.56	0.338	21.9	1.44	0.317
'Therapeutic	10	21.1	1.38	0.294	21.6	1.40	0.303
Toxic	4	20.9	1.07	0.224	21.1	1.03	0.218
Fatal	3	22.8	0.89	0.200	21.3	1.06	0.229

of the control animals are shown in table 1, and the findings in the dogs which received digitalis are given in table 2. For the sake of clarity average values for the four groups of animals are given in table 3. The percentage of solids was practically the same in the dogs which received digitalis as in the controls. The potassium content of the cardiac muscle was markedly decreased in those animals which received toxic or fatal doses of digitalis. The animals which received "therapeutic" doses of digitalis had on the average about fifteen per cent less potassium in their right ventricles than did the controls. However, the left ventricles contained only about three per cent less.

TABLE 4  
*The effect of digitalis on the potassium content of skeletal muscle*

	Animal num ber	Solids per cent	Potassium in dry tissue per cent	Potassium in wet tissue per cent	Remarks
Controls	N <sub>1</sub>	24.9	1.22	0.304	No digitalis
	N <sub>2</sub>	23.6	1.43	0.338	
	N <sub>3</sub>	25.9	1.31	0.339	
Dogs receiving digitalis	D <sub>1</sub>	25.7	1.22	0.313	Received digitalis for eight days Death from digitalis intoxication
	D <sub>2</sub>	24.8	1.24	0.306	

In order to determine whether or not the effect of the drug in regard to potassium was specific for cardiac muscle the skeletal muscle was analyzed in two animals which were given fatal doses of digitalis. In both of these animals the potassium content of the heart was markedly diminished (table 2, dogs D<sub>1</sub> and D<sub>2</sub>) but their skeletal muscles contained normal amounts (table 4). These observations suggest that the action of digitalis on potassium distribution may be specific for cardiac muscle.

#### DISCUSSION

From these data it seems clear that digitalis in toxic dosage causes a diminution in the potassium content of the cardiac muscle of dogs but not of their skeletal muscles, while in amounts corresponding to

therapeutic doses in man this effect is either absent or very slight. Since the majority of the animals which exhibited diminution in the potassium content of heart muscle vomited more or less copiously, the question arises as to whether this diminution might not have been due to the vomiting *per se* rather than to a more direct action of the drug. This appears unlikely because if loss of potassium from the body due to vomiting occurred, one would expect the skeletal as well as the cardiac muscle to be affected. Such was not the case.

It was shown in previous papers (1, 2) that the potassium contents of the cardiac muscle of patients dying of congestive cardiac failure were decreased. Since all of these subjects had received digitalis one might be inclined to think that the drug rather than the disease was the cause of the loss of the potassium. However, doses corresponding to those given the patients caused little or no decrease in the potassium content of the dog's hearts. Furthermore, it was shown that, in patients who received no digitalis, dilatation of the right ventricle due to massive collapse of the lung or to pneumonia was associated with an abnormally low potassium content of that ventricle only, whereas, in dogs digitalis affects the potassium content of both ventricles simultaneously. For these reasons it appears that dilatation rather than digitalis was the chief cause of the changes previously reported in human subjects.

At the present time we are not in a position to understand the significance of the fact that even in therapeutic doses there seems to be a slight tendency for digitalis to diminish the potassium content of the heart muscle. The close relation of calcium to digitalis action is well known (3) (4) and it is conceivable that digitalis may act in such a way as to readjust the ionic balance, even at the expense of loss of base from the muscle. A study of calcium in the cardiac muscle is now being instituted.

#### SUMMARY

The potassium content of the cardiac muscle was markedly less in a series of dogs receiving toxic doses of digitalis than in a group of control animals. Dogs which were given doses corresponding to those used therapeutically in man had a little less potassium in their hearts than did the controls but the difference was slight. The potassium

content of skeletal muscle was not less in dogs receiving digitalis than in control animals

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## THE HEART RATE OF BOYS DURING AND AFTER EXHAUSTING EXERCISE

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In most studies of the reaction of the heart rate to exercise, because of technical difficulties, observations have been made only before and after, but not during the exercise. The cardiotachometer (1) offers a simple means of following the heart rate continuously during periods of rest and violent activity. The action current of the heart is led from the body by two chest electrodes to an amplifier. The amplified current operates a relay system and an electromagnetic counter. With this instrument I have studied the effect of exhausting exercise on the heart rates of 27 boys ranging in age from 9 to 15 years. The wires connecting the subjects with cardiotachometer were 100 feet long, giving them a free range of activity. The exercise consisted in having them run back and forth in a long corridor until they were nearly exhausted, and then up a flight of stairs and down again. The duration of the exercise in the individual cases varied from 2 to 4 minutes with an average of 2½ minutes. Immediately following the exercise the subjects lay down on a bed where they remained until the end of the experiment. The severity of the exertion is shown by the fact that 10 of the boys fell asleep while resting afterwards.

Table 1 presents a summary of the findings. The average of the differences between standing and lying heart rates is 14.6. This corresponds to a difference of 15.7 found by Franke (2) in young men aged 20 to 22. A study of the level of initial pulse rates, of the difference between the rate standing and lying, and of the maximum rate, in relationship to the body habitus, the configuration of the heart as shown by x-ray examination, and the blood pressure, reveals no correlations. In another series of normal cases I have been able to show a similar lack of correlation between heart rate and various bodily measurements (3). The boys were further classified according to a subjective estimate of their physical fitness, and no definite differ-

TABLE I  
*Heart rates before and after exercise*

Caso number	Physical constitution	Age	Weight pounds	Height inches	Physical condi- tion*	Blood pressure mm Hg	Maxi- mum stand- ing per minute	Differ- ence per minute	Heart rate			Time after exer- cise	Rate lying at end of experi- ment per minute		
									Duration of exercise min- utes	Maximum during exercise 1 minute count	15 seconds count per minute				
1	Sthemic	13	113	63	2	110/80	61	72	11	24	164	180	119	68	58
2	Sthemic	13	124	63	3	145/65	81	89	8	24	185	192	111	84	89
3	Sthemic	14	185	68	1		60	78	18	4	179	184	124	90	28
4	Sthemic	13	109	62	1	120/80	89	100	11	2	178	188	99	96	73
5	Sthemic	13	138	62	1	118/70	76	105	29	24	168	172	96	92	40
6	Sthemic	14	145	64	2	100/60	77	117	40	34	190	196	119	83	60
7	Sthemic	13	104	56	2	96/60	72	77	5	24	189	192	120	87	4
8	Sthemic	10	70	52	3	100/70	65	91	26	3	180	192	127	70	2
9	Sthemic	11	106	58	2	110/65	80	99	19	24	185	196	116	79	47
10	Sthemic	13	84 <sup>1</sup>	55	2	128/90	84	92	8	2	201	202	117	104	33
11	Sthemic	15	123	66	1	100/75	69	100	31	24	188	192	123	81	52
12	Sthemic	11	75	54	2	84/60	79	86	2	182	188	109	83	108	
13	Sthemic	13	97	59	2	116/80	79	95	16	24	178	184	105	97	45
14	Hypothenic	13	103	64	2	115/74	64	86	22	4	185	185	121	93	32
15	Hypothenic	13	86	57	2	115/75	85	103	18	34	182	188	103	80	164
16	Hypothenic	9	71	53	3	105/75	87	95	8	34	189	196	102	92	47
17	Hypothenic	10	75	58	2	90/65	83	3	183		188	105	80	53	
18	Hypothenic	12	71	53	3	84/45	72	89	17	2	191	196	124	91	63
19	Hypothenic	13	115	60	2	120/80	73	24	24	197	204	131	94	55	
20	Hypothenic	11	75	54	2	90/70	71	86	15	24	183	183	112	85	

21	Hypothenic	13	98	64	2	105/80	80	97	22	2	178	192	112	96	29	
22	Hypothenic	13	103	63	3	120/60	75	80	19	21	186	188	113	91	67	
23	Hypothenic	13	107	61	2	115/80	61	80	17	21	189	192	131	90	47	
24	Hypothenic	11	80	59	3	98/80	64	81	17	21	170	184	120	80	30	
25	Asthenic	13	91	61	2	110/80	65	88	23	41	197	200	135	98	22	
26	Asthenic	13	72	55	3	100/60	79	105	26	4	185	192	113	72	68	
27	Asthenic	14	106	60	3	110/60	72	99	27	2	154	172	98	90	45	
<hr/>		Average	12.8					74.2	88.0	14.6	21	182.6	190.0	114.9	86.8	51.5

\* 1 poor 2 average 3 excellent.

ence between the initial pulse rates, nor the level reached after exercise in the several groups was noted. Apparently the differences in the degree of training were too slight to manifest themselves in this way.

A striking feature was the very high maximum heart rate shown by most of the boys. The average maximum rate counted for a full minute occurred during the second minute of exercise and was 182.6 or two and one half times the resting rate. The average maximum rate, calculated from the highest rate observed in a fifteen second interval, was 190.0 and occurred on the average 97 seconds after the commencement of the exercise. In three cases the maximum rate was over 200, namely 202, 204, and 200. In adults with severe work the maximum is given usually as from 160 to 170 (4), yet Henderson (5) has observed a rate of 246 after 30 minutes of exhausting exercises which resulted in collapse.

The absolute maximum heart rate attained does not depend on the initial resting pulse rate nor on the duration of the exercise. Others have shown that the rise in heart rate with exercise depends in part on the amount of work performed, and in part on the speed with which it is carried on. In the present study the boys were driven so far as possible to their physiological limit. The differences between them did not manifest themselves so much in the absolute height to which the heart rate rose, as by the time of occurrence of the maximum in each case. The boys who were exhausted after less than three minutes of exercise showed their maximum rate on the average 90 seconds after the beginning of exercise, while those who exercised more than three minutes before having to stop exhibited their maximum rate on the average 120 seconds after the beginning of exercise. This does not necessarily mean that the boys who exercised for longer periods of time were capable of greater exertion, some of them did not put forth the maximum effort of which they were capable until they were repeatedly urged and exhorted.

The increase in heart rate, that is the difference between the lying rate before exercise and the maximum during exercise was on the average 114.9. This increment in rate was the greater, the lower the initial heart rate. Thus those with an increment of over 121 had an average initial rate of 66.1, those with an increment between 110 and 120 had an average initial rate of 75, while those whose increment was

less than 110 had an average initial rate of 81.2. Jaquet (4) has made a similar observation. Part of the acceleration induced by

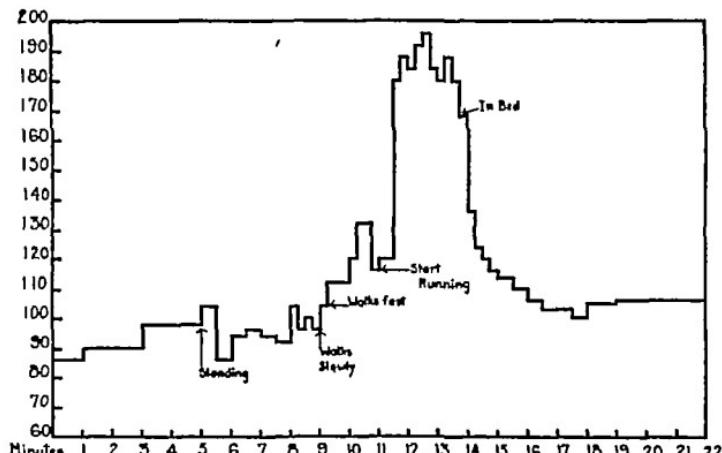


FIG 1 HEART RATE DURING EXERCISE OF CASE 16

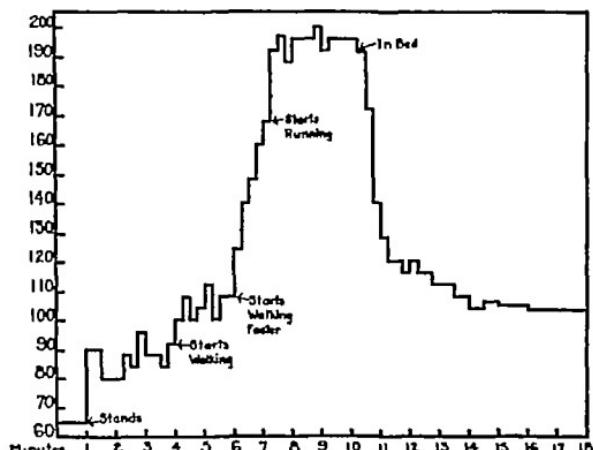


FIG 2 HEART RATE DURING EXERCISE OF CASE 25

exercise is covered by the high initial rate, which already represents a response of the heart to stimuli which raise the rate considerably above the basal

As has been found by Bowen and others (6, 7, 8) the acceleration of the pulse begins immediately with exercise. This is well shown in the curves (fig 1 and 2). Deceleration begins just as promptly, and

TABLE 2  
*Difference between heart rates (lying) before and after exercise*

Case number	Time in minutes after end of exercise									
	1	2	3	4	5	10	15	30	60	90
1	40	31	31	35			31	20	7	
2	53	41	35	33	33	32	28	11	4	3
3	84	57	45	38	38	41		30		
4	42	25		23	23	27	13	16		
5	36	28	22	24	23	22		20	20	
6	50	31		25	26	23	23		6	
7	26	21		15	19	24		17	15	
8	14	5	16	12	14	12		14	11	
9	39	20	25	20		17	16	15	0	
10	62	46		39	35	33	24	22		
11	57	40		29	29	30	33	26	12	
12	57	41	30	25	22	25		21	18	17
13	47	34	33		27		29	24		
14	72	64	48	43	43	42	37	29		
15	31	28	22	21		22		16		
16	37	25	19	13	18	16	17	13	5	
17	22	10	19	17	12	17	12	11	2	
18		62	37	27	28	28	21	20	19	
19	60	51	51	49	41	37	37	35	24	
20	68	29	28	29	29	32	33	16		
21	49	38	33	33	35	28	26	25	24	
22	55	39		32	35	35	34	22	18	
23	48	46	40	43	44	45	42	37	33	
24	61	46	32	29	28	30	30	20		
25	56	51	45	40	40	37	32			
26	67	42		33	27	27	21	16	3	
27	67	41	34	34	40	39	33	21	20	
Average	48 1	30	32 7	29 2	29 5	28 8	27 2	20 7	13 4	10

within the first minute after exercise the major drop in rate is already completed

Much attention has been paid by students of the cardiovascular effects of exercise to the time that elapses after exercise before the pulse rate returns to the resting figures. With light exercise this is

accomplished in two minutes or less. After heavy work the pulse rate may remain high for many hours (4) and a rapid rate may even persist during sleep on the subsequent night (9). In only 5 of 18 of our boys who were followed for a full hour after the exercise did the pulse return to within 5 beats of the previous resting level at the end of an hour. In the whole group the average of the initial lying rates was 74.2, and the average rate at the end of the experiment, which was on the average 51.5 minutes after the completion of the exercise, was 86.8. Thus after almost an hour the heart rate was on the average 12.6 points higher than before the exercise.

In table 2 the differences between the heart rates (lying) before the exercise, and in successive minutes after the exercise are recorded. There is a rapid drop in rate during the first two minutes after exercise, and then a very slow drop of about ten beats in the next half hour, and 7 beats in the succeeding half hour.

We found, as have others, that in the boys who were in better physical condition the reduction in heart rate after exercise occurred a little more rapidly. The duration of the exercise was without visible effect. The closeness with which the heart rate at the end of the experiment approached the rate before exercise was definitely influenced by the level of the original rate. Thus those boys in whom the difference in rate before and after exercise was 15 or less had an average initial resting rate of 77, while in those in whom the difference was over 15 the original rate was 71. The speed with which the heart rate returned to normal was not influenced by the maximum rate that had been reached during the exercise.

#### SUMMARY

Slowing of the heart rate after exercise is so rapid that a true picture of the effect of exercise on the curve of heart rate can be gained only by recording the rate continuously during exercise. Such a study, carried out with the cardiotachometer, on 27 boys subjected to exhausting exercise showed an average maximum rate during exercise of 190. The highest recorded rate was 204. Although the major drop in rate is completed within one minute after exercise, in most cases, the rate is still considerably above the resting level at the end of an hour.

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## HIGH NITROGEN DIETS AND RENAL INJURY

### THE DEPENDENCE OF THE INJURY UPON THE NATURE OF THE NITROGENOUS SUBSTANCE

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An earlier report from this laboratory dealt with the effect upon the kidneys of the white rat, of diets high in protein from different sources. The first diet contained a mixture of seeds supplemented with casein in order to increase the total protein. A second group of rats received diets in which casein was the only protein. Lean beef muscle was the source of protein for the third diet, whereas beef liver furnished the protein in the fourth diet. Our experience with these diets brought out the significant fact that the extent of the injury was largely determined by the nature of the nitrogenous material employed. Thus we were able to state that "Diets containing 75 per cent of dried liver produce a granular kidney in less than one year, but the same amount of casein fed sixteen months, causes only a moderate tubular injury. The effect of a similar amount of beef muscle is intermediate between these two." Since the casein diets in which all (or almost all) of the nitrogen was present as pure protein, caused only negligible injury, and since the beef muscle and liver diets in which a considerable increment of the nitrogen was present in the form of purines and other nonprotein nitrogenous complexes, worked such marked injury, we were led to suspect that the pure protein in a diet does not harm the kidney, but that the nephropathy has its origin in the nonprotein nitrogenous substances present in animal tissues—such as muscle and liver.

In order to test this idea, we fed diets to rats containing 75 per cent of lactalbumin, and wheat and soya bean "gluten" in as large amounts as is consistent with a normal supply of vitamins and salts. These latter two diets contained about 70 per cent of protein. Neither the animal protein (lactalbumin) nor the vegetable proteins, were capable

of producing demonstrable renal injury. At the end of a year's feeding, the urines as well as the histological sections of the kidneys were normal. A further reason for believing that the pure protein of a diet does not originate renal disease, is to be found in the fact that the excretion of large amounts of urea for a long time does not injure the kidney. We added sufficient urea (1) to an otherwise normal diet to produce a nitrogen metabolism equal to that resulting from a diet containing 40 per cent of protein. Other investigators (2) have had a similar experience.

On the other hand, it is quite clear that the ingestion of animal tissues causes the liberation or formation of something that is highly injurious to the kidneys. As already pointed out, diets high in muscle or liver cause fibrotic renal lesions. However, when equal amounts of liver and beef are fed, it is found that liver is much more injurious than beef. In our earlier paper (1) we pointed out that rats invariably died in less than one year when three fourths of the diet consisted of liver, and the kidneys of such animals were granular due to the large patches of fibrous tissue distributed in the characteristic manner.

More recently diets containing lesser amounts of dried whole beef liver had been fed. The damage caused by 60 per cent of liver seemed to be almost as severe as that found in the earlier series of animals that ate the diet containing 75 per cent of liver.

Diets in which the liver made up 40 per cent of the dry weight were more successfully survived by the rats. They were able to grow at the standard rate and at the end of one year, some of them still looked outwardly well. For example, one such female,<sup>1</sup> born January tenth, 1929, was placed on the diet on the ninth of February, 1929, when the body weight was 46 grams. Twelve months later the animal weighed 220 grams. The urine at this time contained 1.6 per cent of albumin and 900 casts per cubic centimeter. The kidneys weighed 2.1 grams. The outer surface was finely granular, grayish brown, mottled with pale gray areas.

Microscopically these kidneys showed the same type of disease as that described for the animals that received the larger amounts of liver (1), but the lesions were, as was to be expected, less extensive.

<sup>1</sup> At autopsy many of our older rats show the chronic purulent pneumonia familiar to other investigators. The lungs of this animal were normal.

The outstanding features in both cases, were the degeneration of tubular epithelium and the patchy fibrosis

When the diets contained only 20 per cent of liver, no evidence of renal injury was obtained

In the search for a more definite cause of this nephropathy, one may think of such a tissue as liver as made up mainly of three types of substances, i.e., (1) the proteins, (2) the large group of nonprotein nitrogenous compounds, and (3) the lipids. Reasons have already been cited for concluding that the renal injury could not be attributed to the proteins. In order to determine which of the other two groups contained the nephrotoxic substances, dried powdered liver was thoroughly extracted with ether and the residue fed. It did not seem necessary to feed the ether soluble fraction since, theoretically, fats are completely oxidized to carbon dioxide and water. Further when such material is incorporated in diets beyond one third of the dry weight, the rats will not eat enough of it to carry out the experiment.

The ether insoluble residue of liver caused the development of kidney disease in the rats that appeared to be entirely analogous to that produced by whole liver. For example, rat 414 ingested a diet containing 50 per cent of liver residue for twelve months, when it weighed 212 grams and gave the appearance of health. The urine contained 2 per cent of albumin and about 5000 casts per cubic centimeter. At autopsy normal lungs were found. The kidneys weighed 2.1 grams and the outer surface was finely granular and mottled. Histologically these kidneys could not be distinguished from those of the rats that received the whole liver.

Since none of the pure proteins that we fed were capable of causing definite kidney injury, and since the liver from which the lipids had been removed produced severe injury, it seemed highly probable that the nephrotoxic substances were to be found in the nonprotein nitrogenous fraction. It will be recalled that liver is much more damaging to the kidneys than beef muscle in equal amounts, and since liver, like other glandular tissues, is rich in nuclear material, it seemed likely that these nitrogenous substances might be chiefly responsible for the injury.

We accordingly prepared the sodium salt of nucleic acid according to the method of Jones (3) and fed it to rats. The sodium nucleate

was substituted for the cornstarch in the following basal diet, in such amounts that the nucleate made up 5, 10 or 20 per cent of the diet respectively

	<i>Basal diet</i>
Lean beef muscle	15
Corn Starch	52 5
Dried yeast	5
Lard	20 5
Salt Mixture (Osborne and Mendel)	4
Cod liver oil	3
	100 0

No kidney injury was detectable in the rats that received the diet containing 5 per cent of sodium nucleate

On the other hand, half of the rats that ate the two higher diets began voiding bloody urine after three to four months, and frequent subsequent inspection of the urines of these animals always revealed gross hematuria. The remaining rats never had macroscopic blood in the urines. Under the microscope the irregular occurrence of red blood cells was recorded. None of the urines contained an abnormally large number of casts. This was the case even when the slightly acid fresh specimens were examined. The absence of casts became the more interesting when it was seen, by means of the microscope, that the nucleic acid was capable of causing extensive renal injury.

A majority of the rats failed to reach the standard adult weight, but the kidney disease can not be attributed to the malnutrition, since the rats that ate the diet containing 5 per cent sodium nucleate had normal kidneys, even though they grew poorly. Furthermore, the animals on the higher diets grew at least as well and showed kidney lesions.

The microscopic study of the kidneys (figs. 1, 2, 3, and 4) showed that the nephropathy produced by nucleic acid is characterized by an extensive formation of fibroblastic tissue and by the hyperplasia of the walls of the arterioles. The amount of new connective tissue was roughly proportional to the concentration of the sodium nucleate in the diet. In the kidneys in which it occurred in the largest amounts, it was distributed in irregular patches and bands throughout the cortex and extended into the medulla. The bands were, however, most fre-

quent in the outer third of the cortex and, by contraction, caused the granular surface evident to the unaided eye

The small arteries of the cortex stood out prominently due to the great thickness of the muscular coat. No abnormality of the intima was noted.

Disbursed irregularly through the areas of connective tissue there were numerous small round cells.



FIG 1 PART OF A BROAD PATCH OF FIBROUS TISSUE THAT EXTENDED FROM THE OUTER THIRD OF THE CORTEX DEEP INTO THE MEDULLA

Several of these large fibrous areas were seen in every section of the kidney.

No definite disease of the glomeruli themselves could be detected. Some of them imbedded in a patch of fibroblastic tissue were compressed and showed thickening of Bowman's capsule. A few fine adhesions were seen.

The convoluted tubules were frequently dilated. Occasionally the dilatation was great enough to produce a cyst-like cavity. The tubular epithelium showed well marked cloudy swelling.

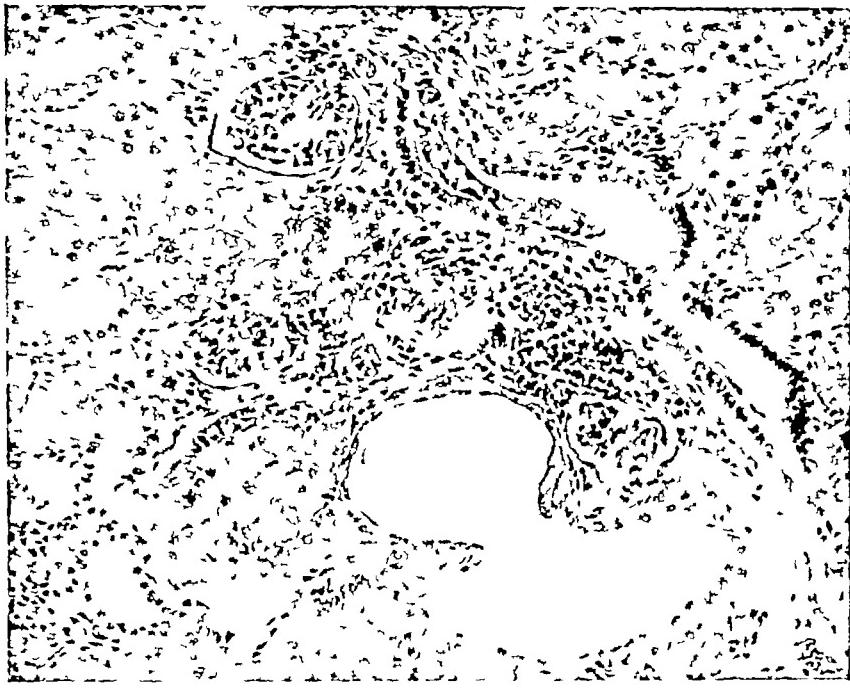


FIG 2 SMALLER PATCHES OF CONNECTIVE TISSUE WERE VERY NUMEROUS

In the center of the field is a greatly thickened arteriole The glomeruli appear to be normal

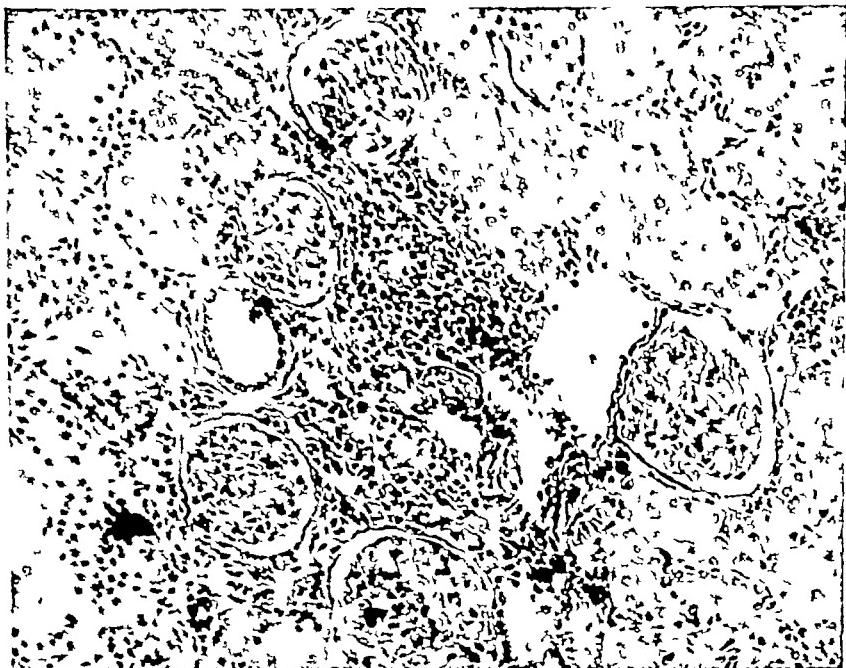


FIG 3 SCATTERED THROUGH THE CORTEX WERE AREAS OF THIS TYPE, IN WHICH MANY SMALL ROUND CELLS WERE IMBEDDED IN THE FIBROUS TISSUE

A thick walled arteriole had been cut longitudinally Cloudy swelling of the tubular epithelium was marked

In the medulla, the tubules contained a considerable amount of calcified coagulum. A few casts were seen.

In the case of the animals that had shown the hematuria, the kidneys, in addition to the above findings, displayed extreme vascular dilatation and very many small and large hemorrhages.

This demonstration that the degradation products of nuclear material are capable of causing kidney disease, brings up the question of the

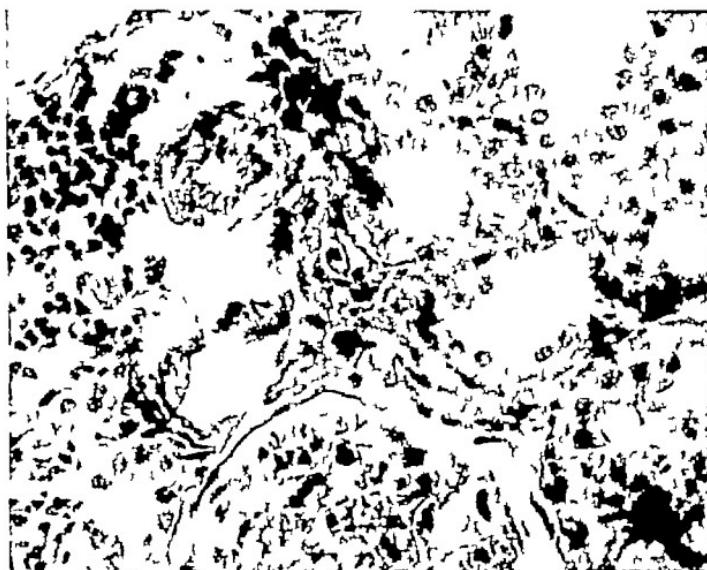


FIG. 4 HIGHER MAGNIFICATION OF AN ARTERIOLE

In the normal rat kidney it is difficult to find arterioles with this magnification because the walls are so thin.

relationship between this fact and the production of nephropathy by animal tissues. The sodium nucleate fed by us contained 13 per cent nitrogen. Theoretically two thirds of it was in the purin form. According to Burian and Schur (4), liver contains 0.12 per cent of purin nitrogen. Dried liver would contain, roughly, three times as much, or 0.4 per cent. A diet made up of 50 per cent of dried liver is definitely nephropathic, and contains only 0.2 per cent purin nitrogen,

whereas the diet lowest in nucleic acid that injures the kidney contains four times that amount of purin nitrogen Furthermore, the microscopic appearance of the lesions is different in the two cases It would, accordingly, appear to be true that liver contains forms of nonprotein nitrogen other than the purins that are nephropathic Since in the case of muscle the purin nitrogen is only half that of liver, it is even more probable that the purin fraction can only be one of the causes of injury

#### CONCLUSIONS

- 1 A diet containing as little as 40 per cent of dried beef liver is nephropathic for the white rat
- 2 A diet containing 20 per cent of sodium nucleate produces a granular kidney in eight months or less Microscopically the disease is characterized by abundance of fibroblastic tissue and by thickening of the media of the arterial wall
- 3 A diet containing 10 per cent of sodium nucleate produces similar lesions of lesser degree

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PROCEEDINGS OF THE THIRD ANNUAL MEETING OF THE  
CENTRAL SOCIETY FOR CLINICAL RESEARCH HELD  
IN CHICAGO, NOVEMBER 21, 1930

*Focal Necrosis of the Liver* By WM J DIECKMANN, M D , St Louis, Mo

Eddington in a report of focal necrosis of the liver as a result of ligation of the common duct and infection with *B. aertrycke*, noted that one of the controls with ligation of the common bile duct, happened to be well advanced in pregnancy. It died four days after the operation and although its liver showed well marked focal necrosis, careful cultural investigations were negative.

We have produced focal necrosis of the liver by feeding meat to dogs and injecting tissue fibrinogen in the peripheral circulation. Lesions produced in this way showed a peripheral hemorrhage and necrosis, the lesion characteristic of eclampsia.

Eddington's experiments have been repeated, using chiefly pregnant guinea pigs near term. Marked liver lesions have been produced, some showing hemorrhage, but the majority pure necrosis. The lesions have been mostly central and mid zonal.

*Untoward Effects of Intravenous Sodium Chloride Solution in Severe Renal Insufficiency* By E G WAKEFIELD, M.D , and N M KEITH, M.D , Rochester, Minn.

Two cases of chronic glomerulonephritis with severe renal insufficiency showed an intolerance to the intravenous administration of sodium chloride. Both had a blood creatinin of 12 mgm per cent on admission. In both, the prolonged intravenous injection of ten per cent glucose solution containing one per cent sodium chloride resulted in anuria or oliguria. Subsequent administration of glucose solution alone resulted in a satisfactory urinary excretion. These findings have both theoretical and practical significance.

*Experimental Edema* By S A SHELBURNE, M D , and W C EGLOFF, M D ,  
(by invitation) and M A BLANKENHORN, M D , Cleveland, Ohio

Edema was produced in a dog by a diet low in protein but adequate in carbohydrate, fat, and vitamin. Edema was also produced rapidly by the selective removal of plasma protein in dogs that were fed the same diet. The chemical, pathological, and clinical findings in these dogs were almost identical and resembled starvation edema (*Der Oedemkrankheit*) more nearly than any other syndrome.

Further evidence of the greater importance of the sodium ion as compared to the chloride ion was added since sodium chloride and sodium bicarbonate increased edema while potassium chloride did not.

Evidence is presented to show that the chloride excreting power of the kidney is normal in experimental edema of low plasma protein origin

*The Renal Lesions in Dogs With Experimental Hypoproteinemia* By LOUIS LEITER, M D , Chicago, Ill

A large series of kidneys has been studied for lesions attributed by other investigators to hypoproteinemia. Various changes, including extensive fibrosis of the kidney, have been observed in the control animals as well as in those undergoing plasmapheresis. There was no correlation between the duration of the hypoproteinemia and the degree of renal pathology. The observed lesions are indistinguishable from "spontaneous" nephritis of dogs. No resemblance exists between renal lesions in dogs and those of so-called nephrosis in man. It may be concluded that the renal lesions of plasmapheresis have nothing to do with nephrotic contracted kidneys in man.

*Experimental Chronic Renal Insufficiency in Dogs, with Special Reference to Arterial Hypertension* By CARL W APFELBACH, M D , and CLYDE REYNOLDS JENSEN, M D , (by invitation), Chicago, Ill

In recent years, there has been an increasing tendency to interpret the clinical manifestations of Bright's disease as part of a general disease of the body, in contrast to the conception formerly held by many, that the alterations in the body were solely due to the changes in the kidneys.

It was deemed worth while, therefore, to develop experimentally a form of renal insufficiency that would be dependent entirely on changes in the kidneys. To accomplish this result, a method was chosen that would injure the glomeruli of kidneys, inasmuch as the severe forms of Bright's disease of human beings are presumably initiated in the glomeruli.

Particles of charcoal, ground and sieved so as to obtain pieces large enough to occlude the glomerular tufts, were injected into the renal arteries of dogs. By this procedure, reduction in kidney tissue was induced without injuring other tissues in the body. It was concluded that the clinical alterations which resulted in the experimental animals revealed the changes that might be expected in human beings as a result of reduction in the quantity of renal tissue. It was further concluded that the application of this syndrome would indicate, in humans, the rôle played by the kidneys in producing the symptoms found in patients suffering from some of the signs of Bright's disease.

There were secured from ninety experimental animals several that lived from several weeks to over one year. A definite group of alterations were observed and these were:

Decrease in the ability of the kidneys to concentrate and dilute urine

Nitrogen retention in the blood stream

Polyuria

Decrease in body weight

Decrease in the carbon dioxide combining power of the blood

Edema and arterial hypertension did not occur

Severe anemia, like that observed in subacute and chronic glomerular nephritis, was not observed

It is concluded, that death cannot be ascribed to renal insufficiency, unless this syndrome occurs

*Further Observations on Climatic Effects, with Particular Reference to Seasonal Alterations in the Human Conception Curve* By C A MILLS, M.D., Cincinnati, Ohio

There will be given a brief review of the previous findings regarding the relationship of climate to such diseases as diabetes mellitus, pernicious anemia, exophthalmic goiter, Addison's disease and angina pectoris. The low mortality from these diseases in the tropics, sub-tropics and orient will be stressed, as well as their high, and increasing mortality rate in the cooler temperate regions.

Since these findings seemed to point to the endocrine system as being most susceptible to climatic influences, it was deemed advisable to see if the sex glands exhibited like effects of climatic stimulation or depression. Monthly birth statistics were collected from various units of population, the months equalized to a 31-day basis, and the births of each month transposed back to the time of conception. The graphs to be shown are thus the "conceptions resulting in live births," with the mean of each month for several years plotted to show the yearly seasonal variations.

The data thus presented leaves no doubt that at least one important climatic factor, temperature, exerts a marked effect on human fertility. The conception rate is highest when the monthly mean temperature is around 60°F, while above 70°F and below 50°F there occurs a progressive depression in fertility. Evidence is not yet at hand to determine which sex is responsible for these effects, or whether both are equally so. Proper control data are presented to rule out certain other possible factors, such as frequency of intercourse, variations in the marriage rate, etc.

*Further Observations on the Effect of Viosterol on Platelets* By LAWRENCE D THOMPSON, M.D., and LILLIAN HADSELL (by invitation), St Louis, Mo.

The increase of platelets following exposure to sunlight has been noted for some time. Phillips and Robertson demonstrated that oral administration of viosterol causes a rise in platelet count in the rat. Recently, announcement was made that the administration of viosterol to normal individuals was followed by a similar rise in platelet count. It was also stated that the platelet count could be markedly increased in various hemorrhagic diseases and that the increase was accompanied by decrease in bleeding and coagulation time but without alteration in the symptomatology or course of the disease. The study has been continued, the series of cases has been augmented. In addition, two other blood dyscrasias have been included.

*A New Method of Interpreting Acid-Base Data* By A BAIRD HASTINGS, M D , Chicago, Ill

By the use of triaxial coordinates an acid-base chart has been devised which permits the evaluation of the acid-base condition of an individual when the pH and CO<sub>2</sub> of the blood is known . Such a chart separates disturbances of the acid-base balance which have their origin in a disturbance of respiratory function from those which have their origin in disturbances of excretory function

The distance which a point on such a chart lies from the normal point of reference and the angle which the line connecting these two points makes with the line of reference can be interpreted as indicating the magnitude of and the qualitative nature of the observed disturbance in the acid-base balance

*Chemical Changes in Blood Plasma and Cells in Intestinal Obstruction, with Special Reference to the Distribution of Phosphorus* By GEORGE MARTIN GUEST, M D , and WILLIAM DE WITT ANDRUS, M D , Cincinnati, Ohio

The changes which occur in the blood plasma during high intestinal obstruction have been well defined, the loss of Cl, and lesser loss of Na, with increase of HCO<sub>3</sub>, being the most noteworthy of the changes Little attention has been paid to the cells, however, and of the blood P only the inorganic fraction has been considered in relation to changes in the acid-base equilibrium

In this study the distribution of blood P was measured as the (1) inorganic, (2) organic acid-soluble, (3) lipid, and (4) total P of oxalated whole blood and plasma Parallel measurements were made of the erythrocyte count, relative cell volume, hemoglobin, total protein, NPN, sugar total base, Na, K, Cl, and CO<sub>2</sub> By means of the relative cell volume, values for the intracellular chemical constituents were calculated and changes in the cell electrolytes were demonstrated which are comparable but not always equivalent to those noted in the plasma electrolytes

In experimental high intestinal obstruction in dogs the total P was increased in both cells and plasma In a typical experiment the total P of the cells increased from 72 mgm per cent to 93 mgm per cent This increase was mainly of the acid-soluble organic P within the cells and, to a less extent, the inorganic P of both cells and plasma It seems likely that this organic P fraction may be actively concerned in the acid-base equilibrium of the cells

*Bacteriologic Studies in Thrombo-angitis Obliterans* By B T HORTON, M D , and A H E DORSEY, M S , (by invitation), Rochester, Minn

The work which we are reporting was started in November, 1927, and has continued for a period of two and one-half years Acutely inflamed veins and arteries were obtained from biopsies or amputations in thirty-four cases of thrombo-angitis obliterans Gram-positive pleomorphic streptococci were obtained in pure culture from nine cases and green producing streptococci from two cases Negative cultures were obtained from arteries and veins from twenty-four normal subjects Various methods of inoculating these organisms into 165 rabbits and three dogs

have been used, and pathologic lesions have been produced in a small number of rabbits, which from the gross and microscopic appearance are identical to those which we accept as that of thrombo-angitis obliterans. This study, though incomplete, suggests that thrombo-angitis obliterans is an infectious disease, and that these organisms may be of etiologic significance.

*Some Clinical Observations on the Use of Theobromine in Peripheral Vascular Disease*

By GEORGE W SCUPHAM, M D Chicago, Ill

The success attending the use of purin base derivatives in angina pectoris suggested its trial in peripheral vascular disease.

Criteria for observation were subjective response, appearance, and measurement of the temperature of the affected extremities under standard conditions by means of a thermoelectric couple.

Observations shown are on typical cases of Buerger's disease, arteriosclerosis with intermittent claudication or tissue impairment, Raynaud's disease, acrocyano-sis, hypertension and normals.

A case of arteriosclerosis with local ischemia observed for more than a year showed definite objective and subjective improvement during periods of medication with a fall in the temperature curves when theobromine was discontinued. In Buerger's disease good results were obtained in the earlier stages, none in the advanced case. There was no demonstrable improvement in Raynaud's, or in acrocyano-sis. Normals showed little effect.

Results seemed to show that theobromine was effective in organic disease of the larger arteries when the symptoms and nutritional changes are best explained on the basis of angospasm.

*Lead Encephalitis* By ROBERT A KEHOE, M D, (by invitation) and C A MILLS, M.D., Cincinnati, Ohio

Experimental and clinical evidence is presented to indicate that the cerebral manifestations of lead poisoning are due to the localization of lead in the brain tissue, rather than in the meninges, or in the blood circulating through the brain.

It is further shown that lead forms a stable and insoluble compound in the brain under the customary conditions of physiological activity. This suggests that such lead as is brought to the brain through the circulation is quantitatively bound.

Certain tentative conclusions as to the conditions under which saturnine encephalopathy develops, and certain suggestions as to the treatment of lead poisoning in such a manner as to spare the nervous tissues, arise from these considerations.

*The Relationship Between Oxygen Consumption and Nitrogen Metabolism in Pernicious Anemia and Leukemia* By C W BALDRIDGE, M D, and A P BARER, Ph D, (by invitation), Iowa City, Iowa

In pernicious anemia and in leukemia a definite relationship between oxygen consumption and nitrogen metabolism has been demonstrated. During periods of rapid cell destruction especially if there be a negative nitrogen balance (relapse in

pernicious anemia or soon after roentgen ray treatment in leukemia) the oxygen consumption is increased. The consumption of oxygen decreases while nitrogen is being stored in spite of the fact that young cells are being formed rapidly (e.g., during remissions in pernicious anemia or several days after roentgen ray treatment in leukemia). The total oxygen consumption would probably be more increased after roentgen ray treatment were it not for the loss in the urine of incompletely metabolized protein.

*Observations on Some Conditions Causing R-T Deviations Characteristic of Coronary Occlusion* By LOUIS N KATZ, M D, Chicago, and A W WALLACE, M D, (by invitation), Cleveland, Ohio

Recent observations by Scott, Katz and Feil have shown that pericardial effusions will lead to R-T deviations characteristic of coronary occlusion. Animal experiments have shown that ligation of a coronary artery will not lead to such R-T changes until cardiac incompetence sets in (Feil, Katz, Moore and Scott). Three cases are presented in this report to illustrate the rôle cardiac incompetence plays in producing the characteristic R-T deformities. One case shows the effect of cardiac incompetence superimposed on an acute coronary thrombosis. The second shows the effect of cardiac incompetence in the presence of coronary sclerosis but with no thrombosis present. The third shows the effect of acute myocardial incompetence added to a slight pericardial effusion. In all three cases typical R-T deviations are present. In each the cardiac incompetence was the primary and major cause for these electrocardiographic changes.

*Interpretation of the Initial Deflections of the Ventricular Complexes of the Electrocardiogram* By A G MACLEOD, M D, PAUL S BARKER, M D, and F N WILSON, M D, Ann Arbor, Mich

Obviously, the electrocardiogram recorded in the three standard leads must be the resultant of many opposed, or partially opposed, potential differences which occur in the heart during the inscription of the QRS complex. It must, therefore, be that only those forces which are unopposed, or only partially opposed, can produce measurable potential differences at the surface of the body. By applying these theoretical conceptions to a detailed anatomical study of the canine and human heart it is possible to show that the results of Barker, Macleod and Alexander, who believe that what is, at present, called right bundle branch block, is in reality left, and vice versa, are the ones to be expected. It is also possible to reinterpret the observations of Lewis so as to show that the above authors' views are in conflict only with Lewis' interpretation of his data, and not with the data itself. Rational explanations of bundle branch block curves and preponderance curves can be made on this basis.

*The Treatment of Patients with Addison's Disease with the Extract of the Suprarenal Cortex Prepared by Swingle and Pfiffner* By LEONARD G ROWNTREE, M D, and CARL H GREENE, M D, Rochester, Minn

An extensive experience in the use of the so-called Muirhead regimen in the treatment of patients with Addison's disease has convinced us of the futility of ordinary therapeutic measures in combating the crises of acute adrenal insufficiency, and also of the great need for a more active cortical preparation which could be administered either hypodermically or subcutaneously. In March, 1930, Swingle and Pfiffner announced the preparation of an aqueous extract of the supra renal cortex which would maintain the life of bilaterally adrenalectomized cats indefinitely, and later they reported that by the administration of this extract they were able not only to revive comatose animals, prostrate and on the verge of death from adrenal insufficiency, but also to restore them to a normal condition and to keep them in perfect health. The importance of such an announcement and the interest aroused by the possibility of using this extract in clinical medicine is obvious.

The preparation which we are using now is almost free from epinephrine, is suitable for intravenous administration and is almost nonirritating locally. We have used this in the treatment of six patients with Addison's disease. The results in these cases convince us of the efficacy of this cortical hormone. The disappearance of anorexia, increase of appetite, the gain in weight and the definite euphoria were striking in all cases. As long as the preparation could be administered, the results were all that could be desired. Our supply of the preparation has been extremely meager and intermittent, so that we have not been able to observe the results of consistent dosage and continued administration, but the immediate results in a crisis are excellent. The disease, however, is chronic, and it will be necessary for several years to elapse before a final appraisal can be made of the value of this cortical hormone in Addison's disease.

*The Intravenous Administration of Barium Chloride in Humans* By J H FOULGER, M.D., (by invitation), and JOHNSON MC GUIRE, M.D., Cincinnati, Ohio

Considerable interest has been aroused by the work of Cohn and Levine on the use of barium chloride in cases of heart block with Stokes-Adams attacks. Several papers on the subject have appeared subsequently, but the results have been variable and the drug has hitherto been given exclusively per os. The slowness of its absorption and perhaps in some cases the almost complete or complete lack of absorption might account for its failure. The desirability of securing therapeutic effects promptly in Stokes Adams syndrome is apparent. Therefore, it seemed of interest to study the effect of intravenous administration of the drug, using, of course, a much lower dosage than that advised orally.

The characteristic effect on the human and animal heart were studied electrocardiographically, and these findings are described.

*A Study of Five Hundred Diabetic Patients* By ELMER L SEVRINGHAUS, M.D., Madison, Wis.

These patients, seen in the eight years since insulin has been available, have had insulin only when necessary to control the glycosuria, (or to maintain blood sugar

levels more nearly normal in a few younger individuals) Diets have been planned according to Woodyatt's rule,  $F = 2C + 0.5P$  The maintenance standard has been at the accepted normal weight for the patient, and with no limitation on occupation Under such conditions 57 per cent required insulin at discharge from the hospital

Ketosis was observed in the first urine examined in the hospital in 58 per cent of the patients Insulin was required at discharge by  $\frac{1}{2}$  of this group Of the 42 per cent without ketosis, only  $\frac{1}{2}$  required insulin In no case of this latter group was the insulin large Therefore, the occurrence of ketosis with glycosuria at the time of the first examination may be accepted as a prognostic indication that insulin will be needed, although later improvement may render its continuance unnecessary This information saves time in planning treatment

Of the 500 cases 90 are known to have died Causes, which are determined in 75 of these, include, in order of importance sepsis, cardiovascular-renal disease, coma, and carcinoma Septic processes include, in the following order pneumonia, gangrene, tuberculosis, gas bacillus infection after amputation, and a miscellaneous group In ten of the cases neglect on the part of the patient or parent after instruction and discharge from the hospital is directly responsible for death No uncomplicated case of coma has been lost in the hospital

In the entire series, routine histories reveal reduced or absent patellar reflexes in 45 per cent Pains in the extremities (neuritis?) are recorded in 34 per cent These pains include cases in which vascular conditions are probably primarily at fault There are 37 per cent of these cases with visual disturbances, excluding cataracts and refraction errors acquired before diabetes This visual error is often relieved by the diabetic therapy itself In any case, lens fitting should not be undertaken within less than one month after the patient is sugar free

*A Study of Blood Phosphate and Blood Glucose Curves After the Oral Administration of 100 Grams of Glucose* By HENRY J JOHN, M D , and D ROY McCULLAGH, Ph D , (by invitation), Cleveland, Ohio

The curve of phosphate and glucose after the administration of 100 grams of glucose orally to sixty patients has been studied The statement that they are related is confirmed, but we find that the so-called typical diabetic PO<sub>4</sub> curve is not always in itself diagnostically reliable If we depend upon phosphate curves alone for diagnosis an error of 19 to 67 per cent is incurred, depending upon the condition of the group under consideration In hyperthyroidism the errors are from 40 to 67 per cent

The excretion of phosphates bears no relation to the urine output so that we can consider that the phosphate excretion is not a pure mechanical washing out of phosphate, depending on the increase of urine excretion, but a function of a metabolic process, totally independent of the amount of urine excreted In diabetics the phosphate excretion is markedly increased

*Diabetes Mellitus Associated with Addison's Disease (Report of Two Cases)* By

FRANK N ALLAN, M.D., Rochester, Minn

There has been much speculation regarding the relationship of various endocrine glands to diabetes, and the rôle of the adrenals has received especial consideration. The theory that diabetes might be due to excessive activity of the adrenals was formerly strongly supported. Recent investigations showing that continuous administration of epinephrine may cause suppression of oxidation of glucose have revived this idea.

Two cases of diabetes associated with Addison's disease are reported. This combination represents a rare clinical picture. It also suggests various problems of theoretical interest.

*Jerusalem Artichokes and Liver in the Treatment of Diabetes Mellitus* By SAMUELSOSKIN, M.D., and H. F. BINSWANGER, M.D., (by invitation), and SOLOMON  
STROUSE, M.D., Chicago, Ill.

The experimental use of Jerusalem artichokes and of liver in the treatment of diabetes mellitus is reported. No evidence was obtained that these substances are of any therapeutic value in this condition. Each patient was hospitalized throughout the experiment, and judgment was chiefly based on daily urinary sugar and nitrogen excretion over adequate periods rather than on temporary changes in blood chemical values or alterations in respiratory exchange. Adequate control periods on orthodox diets, with or without insulin, were instituted in all cases, the artichokes or liver being then substituted in the diet so as to leave the caloric intake and the distribution of calories between protein, fat and carbohydrate unchanged. The results of other workers are discussed in the light of the work here presented.

*Quantitative Relationships Between Dosage and Therapeutic Response in 100 Patients**with Pernicious Anemia Treated with Liver Extract of Desiccated Stomach*

By CYRUS C STURGIS, M.D., and RAPHAEL ISAACS, M.D., Ann Arbor, Mich.

The rate of response to therapy was observed in 50 patients with pernicious anemia treated with liver extract and 50 similar patients treated with desiccated stomach. The average maximum reticulocyte percentage reached in the former group was 22.36 per cent (calculated 23.43 per cent) and in the latter group 22.63 per cent (calculated 22.58 per cent). The maximum reticulocyte percentage was reached in 6.9 days by the liver treated group and in 7.84 days by the stomach treated patients. This is attributed to the slower absorption of the non-soluble desiccated stomach. A suboptimal dose, infection, gastric retention, or blood transfusion decreases the observed maximum reticulocyte percentage below calculated height. A massive dose decreases the latent period and increases the height of the reticulocyte rise. Within limits, the larger the dose, the shorter time required for the red blood cell count to reach normal.

*Chronic Ulcerative Colitis (Further Studies)* By M. H. STREICHER,<sup>1</sup>

(by invitation), and C S WILLIAMSON, M.D., Chicago, Ill.

Until recently, all reports on the subject of ulcerative colitis were discouraging. For many years it has been the opinion of most gastroenterologists that the treatment of colitis was mainly that of the specific forms of ulcerative colitis and that the diagnosis of the nonspecific ulcerative colitis was based on the existence of colon ulcers with negative bacteriologic observations.

*Method of procedure* In the past three years we have carried on a careful survey of all cases of diarrhea observed in our gastro-intestinal clinic, first, to confirm or refute the observations of Bargen and, secondly, to determine the specificity and therapeutic value of autogenous vaccine. The patients were properly prepared a day before and routine proctoscopic examinations were made in each patient. Cultures were then obtained through the proctoscope by means of a sterile swab and transferred at once to a proper medium for incubation and subsequent culture and subculture. The individual strains of bacteria were then isolated from the cultures and a polyvalent vaccine was prepared. The autogenous vaccine was then administered hypodermically to the patients in progressively increasing dosages in accordance with the severity of the infection.

*Results* Briefly summarized, the results clearly demonstrate that most remarkable improvement was noted in about 80 per cent of the cases observed.

Our results show that in most cases of ulcerative colitis green-producing gram-positive diplococci are found predominating. Frequently these are in association with other bacterial flora.

In addition to the green-producing gram-positive diplococci and hemolytic streptococci, we found other bacteria in ulcerative colitis such as diphtheroid bacilli, *Staphylococcus albus* and *aureus*, *Staphylococcus albus-hemolyticus*, *Bacillus pyocyanus*, *B. lactis-aerogenes* and *B. coli*.

*Treatment* The method of approach in general was three-fold, namely, prophylactic, dietary and specific. In reference to diet, one is impressed considerably with the encouraging results obtained with an essentially high calory, high vitamin and low residue diet. This specific treatment consists of injections of polyvalent autogenous vaccine hypodermically.

*Conclusions* (a) The green-producing gram-positive diplostreptococcus is not the sole etiologic factor of chronic ulcerative colitis.

(b) A polyvalent autogenous vaccine given hypodermically is of considerable therapeutic value.

(c) The green-producing gram-positive diplococci are all inulin negative and the majority are mannite negative.

*Response of Secondary Anemia to Fetal Liver* By CHARLES H. WATKINS, M.D., and HERBERT Z. GIFFIN, M.D., Rochester, Minn.

In an article on the treatment of secondary anemia by Herbert Z. Giffin and Charles H. Watkins read before the Section on Pharmacology and Therapeutics at the Annual Session of the American Medical Association, Detroit, June 26, 1930, a review of the clinical results following the administration of desiccated fetal liver in the treatment of 120 cases of chronic secondary anemia was presented. The

paper submitted for presentation before the Central Society for Clinical Research is an elaboration of these data and an evaluation of the results in various types of secondary anemia as classified both from the clinical and morphologic standpoint.

*Scalenotomy in the Surgical Treatment of Pulmonary Tuberculosis* By JOSEPH W GALE, M.D., (by invitation) and WM S MIDDLETON, M.D., Madison, Wis

Surgical measures for the treatment of pulmonary tuberculosis involve two fundamental principles, namely physiologic rest and compression of the involved lung. The first of these indications has been met in phrenicotomy and intercostal neurectomy, which bring about paralysis of two of the most important groups of respiratory muscles. The scalene group alone of the important muscles of respiration has escaped surgical attention.

The sound clinical and experimental observations of Hoover established the respiratory function of the scaleni. By fixing the first rib they permit of an advantageous action of the upper three intercostal groups on their attached ribs with cephalad movement of the same. Paralysis of the scaleni is attended by a caudad movement of the upper three ribs on inspiratory activation of the intercostal muscles.

Because of the intercostal overactivity attendant upon phrenic paralysis the concurrent division of the scaleni was suggested to induce apical rest. The anatomic relationships render the combined operation of phrenic block and scalenotomy quite easy. The operation has been completed in a small series of cases, both alone and in combination with phrenic block, without increased reaction or disability and with gratifying results. The indications are discussed.

*Analysis of One Hundred and Five Electrocardiograms Showing Changes Associated with Coronary Thrombosis* By WALTER S PRIEST, M.D., (by invitation), and W W HAMBURGER, M.D., Chicago, Ill

Those changes commonly considered indicative of coronary thrombosis are tabulated according to the lead or combination of leads in which they occur. They are inversion of T, positive S-T interval, high take-off of T, deep Q, distortion of the isoelectric line. In addition slurring and notching of QRS, occurrence of positive S-T without inversion of T, axis deviation, changes in axis deviation during a series of records, low amplitude of QRS deflection, and abnormalities of the P wave noted. The relation of these changes to the clinical findings is then analyzed. For this purpose the patients are grouped clinically into five classes.

(1) Those with undoubted signs of coronary thrombosis, (2) those with anginal attacks but without classical signs of thrombosis, (3) those without history of pain but with other cardiac symptoms, (4) those without pain or other cardiac symptoms, and (5) those without cardiac symptoms but with upper abdominal lesions. Patients with upper abdominal lesions and cardiac symptoms, and patients with syphilis are considered under groups 1 to 4 as indicated. Autopsy findings are included when available.

Only 39 per cent fell in group 1, 33½ per cent fell in group 2, 9 5 per cent in group

3, 16 per cent in group 4, and 19 per cent in group 5. Approximately 10 per cent of those in group 1 had associated upper abdominal lesions. 57 per cent of those in group 2 had associated upper abdominal lesions. One patient in group 2 and one in group 4 had syphilis.

*The Effect of Lugol's Solution on the Elevated Basal Metabolism in Conditions Other than Exophthalmic Goiter* By HARRY B FRIEDGOOD, M D, (by invitation) (Introduced by Cyrus C Sturgis, M D), Ann Arbor, Mich

The effect of Lugol's solution in exophthalmic goiter suggested a similar investigation in other diseases characterized by an elevated oxygen consumption, notably, leukemia, polycythemia vera and acromegaly.

Symptomatic improvement occurred in all of the eight patients with chronic lymphatic leukemia and three of four patients with chronic myeloid leukemia. Aleukemic leukemia (3) and the acute leukemias (2) did not respond similarly.

Basal metabolism and pulse rates were significantly decreased in three fourths of the patients (ambulatory) with chronic lymphatic leukemia, and in one of four patients with chronic myeloid leukemia. Polycythemia vera (1) and acromegaly (1) also exhibited favorable decreases. Initial basal rates recurred under continued treatment as in exophthalmic goiter, but sometimes the depressed pulse rate lagged behind the increasing metabolism. Acute exacerbations, severe anemia and previous roentgen therapy inhibited the usual decreases. Following treatment, the leukocytes in chronic lymphatic leukemia appeared temporarily depressed, but no relation existed between rate of metabolism and leukocyte counts. Eye signs of exophthalmic goiter occurred in some of the patients with chronic leukemia.

The similarity of response to iodine suggests a non-thyrogenous disturbance, responsible for the elevated basal metabolism, and common to exophthalmic goiter, chronic lymphatic and myeloid leukemia, acromegaly and polycythemia vera.

*Studies in the Circulation. Injection Method Preliminary Report on Clinical Cases* By J MURRAY KINSMAN, M D, JOHN WALKER MOORE, M D, and W F HAMILTON, M D, (by invitation), Louisville, Ky

Our method for determining the output of the heart, circulation time and the amount of blood in the heart and lungs and the total blood volume has been applied to certain cases of hyperthyroidism, pernicious anemia and heart disease, both during and after recovery from the acute manifestations.

*Observations on the Clinical Course and Effects of Treatment in 112 Cases of Portal Cirrhosis with Ascites* By C B CHAPMAN, M D, (by invitation), L G ROWNTREE, M D, and A M SNELL, M D, Rochester, Minn

The serious prognostic significance of the development of ascites in patients with portal cirrhosis is shown by the fact that 52 per cent of this group of patients were dead within sixteen months after the ascites appeared. It would seem that the appearance of ascites may be regarded as evidence of failing compensation in the

portal circulation and that ascites has somewhat the same significance in portal cirrhosis that it has in cardiac disease. Treatment with diuretics is ordinarily effective in the control of ascites early in the course of the disease, but it becomes less so as time progresses. There is no type of treatment which seems to be effective in the control of the hepatic lesion itself.

*Non Specific Protein Therapy in Duodenal Ulcer* By LEON SCHIFF, M.D., and R J NORRIS, M.D., (by invitation), Cincinnati, Ohio

The effect of intramuscular injections of Aolan (purified milk protein) in 27 cases of duodenal ulcer are reported.

Of these cases, 20 were ambulatory and 7 hospitalized. Those hospitalized were with one exception (case with hematemesis) not confined to bed and given a general diet. No medication was prescribed.

Marked improvement or practically complete symptomatic relief occurred in 18 or 66.7 per cent, and less marked though definite improvement in 5 or 18.5 per cent, making a total of 85.2 per cent deriving benefit.

Improvement was relatively more frequent in the colored than in the white individuals.

Improvement seemed to occur more frequently in those with normal acidity than in those with hyperchlorhydria.

A second course of injections in some instances of relapse was equally as effective as the first.

Striking gain in weight was at times noted.

Improvement seemed to occur less frequently in the presence of ulcers.

No change in the degree of acidity or volume of gastric juice was noted, using the method of Bloomfield and Keefer.

Patients with symptoms of less than a year's duration obtained complete relief.

*The Influence of Ethyl Alcohol Upon the Gastric Absorption of Phenol in Rabbits*

By F LOWELL DUNN, M.D., and ANNE M PERLEY, (by invitation), Omaha, Neb.

The value of alcohol lavage in phenol poisoning has been repeatedly questioned. The development of blood phenol methods permits a study of the rate of absorption of phenol with and without the addition of alcohol. These studies were made on rabbits. The free blood phenol showed a somewhat more rapid rise in a group of rabbits receiving phenol and alcohol than in a group receiving phenol alone, but the final levels were essentially the same. The conjugated phenols showed only a very slight rise.

*Electrocardiographic Changes Produced by Experimental Occlusion of Coronary Vessels in the Dog* By A R. BARNES, M.D., Rochester, Minn.

Experiments investigating the electrocardiographic changes resulting from occlusion produced by ligation of various branches of the coronary arteries in dogs have been carried out. Contrary to the previous investigations it appears that

ligation of a branch of the right coronary artery producing infarction in the right ventricle produces electrocardiographic changes which are characteristically different from those obtained when a branch of the left coronary artery is ligated

Infarction produced in the posterior basal portion of the left ventricle in dogs does not produce electrocardiographic changes similar to those obtained following infarction in a like region in the human heart. Except for that difference it seems possible to correlate the results to date with electrocardiographic changes in man following acute coronary occlusion as described by Barnes and Whitten. It should be noted that the operative procedure and incision of the pericardium involving a possible change in the axis of the heart must be taken into account in interpreting these results and this factor has not been completely evaluated as yet.

*Parasystole—A Study in the Origin and Conduction of Ectopic Impulses of Supraventricular Origin* By JULIUS JENSEN, M.D., (by invitation), and DREW LUTEN, M.D., St. Louis, Mo.

Cases of so-called nodal rhythm which show premature ventricular beats following P-waves are rare. We have studied six cases in detail. Evidence is presented that these premature beats originate in ectopic foci and interfere with the dominant "nodal" rhythm. These ventricular responses to more than one focus constitute parasystole. The conditions of parasystole with supraventricular pacemakers are studied, consideration being given to rhythmicity, rate and location of the foci, also to the block protecting the upper pacemaker and to the conditions under which this pacemaker acts on the ventricle. The probability of more than one "nodal" pacemaker in certain instances is discussed.

The hypothesis of reciprocal beating is considered, but evidence against such an explanation of our cases is presented. Most of the cases in the literature described as reciprocal rhythm fulfill the requirements of parasystole and the conception of reciprocal rhythm in any case must still be held subjudice.

*Carcinomatous Bone Metastases Without Roentgen Evidence (Special Reference to the Spinal Column)* By E. L. TUOHY, M.D., Duluth, Minn.

There is a general assumption and much developing proof that the more common carcinomas metastasize at an early period to certain important bony sites. The clinical search for evidence of this disastrous bony invasion has called into use an earnest effort to show the extent and degree of such invasion through roentgen films. In prosecuting this search it has been clearly found that the roentgen changes are those produced by so-called osteoblastic changes through extra calcium deposition, and the converse, or osteoclastic changes incidental to bony replacement by soft tissue. That the prostate cancer has the faculty of setting up osteoblastic changes, and hence the creation of exaggerated calcium deposits within the bone, no one familiar with the process will longer doubt.

Hypernephroma (more recently known as carcinoma of the kidney) has the subtle faculty of invading the bones, and to a very notable degree those of the head. Carcinoma of the thyroid, as also carcinoma of the bronchus, not uncom-

monly invades the cranial cavity as well as the cranial bones, practically all cancers, including the stomach and uterus may metastasize into the vertebrae. Hence, the vertebral column is recognized as a crucial zone in terms of metastases, prognosis and operability.

For the purpose of this report the actual spinal column and corresponding films are shown. In three cases, though the vertebrae were badly seeded with metastases (from prostate, stomach and cervix, respectively), the roentgen evidence is negative. The sections will show that when the films were taken the process was far advanced.

*Conclusion:* Negative evidence of bony metastases (especially in spine) does not rule out marked invasion. In the problem of cancer control some much more subtle evidence of local and systemic invasion must be sought than any method of visualization now possible.

*Surgical Operations and Associated Infections as Possible Etiological Factors in the Development of Exophthalmic Goiter and Hyperthyroidism from Adenomatous Goiter* By WILLIAM A PLUMMER, M.D., and CHARLES MAYO, 2ND, M.D., (by invitation), Rochester, Minn.

From records of The Mayo Clinic we collected thirty eight consecutive cases in which surgical operation on some part of the body other than the thyroid gland preceded partial thyroidectomy for exophthalmic goiter or for hyperthyroidism from adenomatous goiter. Both operations were done in The Mayo Clinic. We were not particularly interested in the fact that the patients had been subjected to an operation previous to thyroidectomy except for the fact that the previous operation had caused a record of their condition to be made and that this record could be compared with that of a later time when they had demonstrated hyperthyroidism. We wished to learn if the records of the patients made during their first stay in hospital contained evidence to indicate that acute or chronic infectious processes or other factors associated with the first operation might have been etiologically related to the development of exophthalmic goiter or hyperthyroidism from adenomatous goiter. In those cases in which there was relationship in time between the development of the toxic symptoms of goiter and the first operation, the element of mere coincidence can not be excluded. Our investigation, however, tends to support the contention that infection may be an aggravating or precipitating factor in the development of exophthalmic goiter or hyperthyroidism from adenomatous goiter, in certain cases.

*Basal Metabolism and Specific Dynamic Action of Foodstuffs in Normal Pregnancy* By F. LOWELL DUNN, M.D., and HELEN WYANDT, (by invitation), Omaha, Neb.

The respiratory gas exchange and urinary nitrogen was determined at two weeks to monthly intervals during the course of two normal pregnancies, and the puerperium. The basal metabolism data conform to the case of Sandiford and Wheeler. The specific dynamic action for carbohydrate, protein, and fat was

determined at the same intervals. Essentially normal values were obtained throughout although the dynamic action of fat shows a steady diminution in the two cases.

*Nutritional Edema* By ALEXIS F HARTMANN, M D , and MILTON J SENN, M D ,  
(by invitation), St Louis, Mo

The composition of the plasma in cases of nutritional edema has been studied from the standpoint of (1) total and fractional protein concentration, (2) colloidal osmotic pressure, and (3) total acid-base equilibrium. A comparison is made with other types of edema as regards such findings, and the response to transfusions and high protein diet.

*Sporadic Septic Sore Throat Due to Streptococcus Epidemicus* By I PILOT, M D ,  
Chicago, Ill

*Epidemicus* septic sore throat is a well established clinical entity due to the *Streptococcus epidemicus* (Davis), and is spread through milk from the udder of a cow infected by this organism. In the isolation of this streptococcus certain characteristics can be brought out by the use of blood agar enriched by ascites fluid, which differentiates the *streptococcus epidemicus* from other hemolytic streptococci. The use of this medium has led to the ready recognition of carriers and of sporadic cases of septic sore throat. Several such patients are reported, and in addition complications such as otitis media, mastoiditis, meningitis adenitis, empyema, septic arthritis are found in other patients to be due to this streptococcus, which was often also demonstrated in throat cultures. These streptococci produce exotoxin which in skin tests with filtrates and neutralizing serums differs from the toxin of *Streptococcus scarlatinae*.

*The Danger of Overdosage with Parathormone* By WALTER M BOOTHBY, M D ,  
Rochester, Minn

Greenwald and Gross, in 1925, showed that the excretion of calcium in the urine was markedly increased following the administration of large doses of parathormone and sounded a warning as to the possible danger of the use of too large doses in parathyroid insufficiency.

Since then the clinical use of parathormone has become quite extensive and the doses that have been reported vary between 20 and 160 units daily.

We have studied one case in which the use of 150 units for four days was demonstrably harmful. Other patients studied have shown that in cases of chronic parathyroid insufficiency where the use of parathormone must be continued for a long time and probably indefinitely the size of the dose should probably not exceed 5 or 10 units daily.

*The Action of Soap on the Animal Organism.* By IRVINE H PAGE, M D , (by  
invitation), and E V ALLEN, Rochester, Minn

A detailed study was made of the effect of injection of soap on white rats, white mice, and rabbits. It was found that soap injected intravenously or intraperitoneally produced extremely toxic conditions and a variety of pathologic changes in the tissues. Chemical analysis of the livers and kidneys showed that in general the total fat was decreased and the iodine number increased. Pharmacologic studies disclosed that the soap lowered blood pressure, stimulated respiration, produced cardiac irregularities and increased the permeability of the membranes to dye. The physiologic significance of these experimental observations were considered.

*Electrocardiographic Studies on Changes in the Position of the Heart* By M H NATHANSON, M.D., Minneapolis, Minn

It has been noted by several observers that the form of the electrocardiogram is altered on change in position of the body, as a result of a shift in the position of the heart. It has been suggested that the absence of such a change may be considered as evidence of adhesive pericarditis, a condition difficult to diagnose. When an individual turns from the reclining position to one side and then the other, there is a rotation of the heart about two axes, (1) antero-posterior, and (2) longitudinal. The electrical effects of these two types of rotation are in the opposite directions and it is theoretically possible that one may neutralize the other, thus affecting no change on the electrocardiogram.

A group of normal individuals and cardiacs in whom a fixed heart was very improbable was studied and a detailed analysis made of the electrocardiographic alterations on change of position and on different phases of respiration. A change in the amplitude of the QRS, usually in the first and third leads was practically constant, but a variety of combinations of changes was noted. The most frequent alteration was a change in the direction of right preponderance when the individual was turned on the left side, with a tendency to left preponderance on turning to the right. The effect of such factors as habitus of the individual, size of heart and the presence of pleural adhesions was noted. Observations were also made on three cases of adherent pericardium.

*Radical Inclusions of Giant Cells* By EDWIN T HIRSCH, M.D. Chicago, Ill

Visceral and focal lesions resembling tuberculosis and containing giant cells with radial inclusions are mentioned in fourteen reports now on record. The nature of these inclusions is an enigma, and a wide variety of opinions have been expressed as regards their origin and the significance of the associated lesions. Visceral lesions of this kind in tissues obtained postmortem from the bodies of four patients and focal lesions in twenty other tissues removed surgically, are the human tissues that furnish the basis of my report. The results of the chemical analysis of human tissues and those of experiments in animals suggest that the inclusions are modified saturated fats.

*Experimental Production of Arteriosclerosis in the Aorta and Coronary Arteries*  
By DON C SUTTON, M D , and M D DAVIS, M.D , (by invitation), Chicago,  
Ill

During the past year, in a group of some fifteen or sixteen dogs, the ramus descendens branch of the left coronary artery has been traumatized, the dog sutured and allowed to live. For a period of 200 days or more these dogs have been on a high calcium intake, with doses of parathormone. In this group there have developed arteriosclerotic changes in the aorta, scar tissue around the ramus descendens branch and diffuse scar tissue in the myocardium. Cardiac measurements have been made by x-ray and electrocardiographs have been taken at intervals during the course. The final conclusions of all this work will be based on autopsy findings.

*Albuminuria in Young Men* By H S DIEHL, M D , and C A McKINLAY, M D ,  
Minneapolis, Minn

The frequency of albuminuria is noted in approximately 20,000 young men who entered the University of Minnesota since 1921. In part of the series, different tests for albumin were used and are compared. On the first examination, 5.24 per cent were found to have albumin, two-thirds of these later had negative reports. Only 6.74 per cent had what seemed to be definite evidence of kidney disease. Under proper control, the group with albuminuria was studied as to age, height-weight percentage, pulse rate, blood pressure, histories of certain past diseases and the condition of the nose and throat. The changes noted, compared to a control group, were a slight reduction in average age and in mean height-weight percentage and a definite increase in the incidence of acute upper respiratory tract infections, of chronic nasal discharge and of abnormal tonsillar conditions.

*Auricular Fibrillation as the Only Manifestation of Heart Disease* By W M FOWLER, M D , (by invitation), and FRED M SMITH, M D , Iowa City, Iowa

Ten patients are recorded, in whom the auricular fibrillation was the only evidence of cardiac disease. The oldest was 34 years of age. In one, the irregularity was attributed to stimulation of external auditory canal, one to exertion, four to alcohol, one to carbon monoxide, one to ether anesthesia, and two were attendants in gasoline stations.

In seven, the auricular fibrillation was paroxysmal, while in three, it had persisted from two weeks to two and one-half years. The sinus rhythm was restored in the latter three by quinidine. The patient with the auricular fibrillation of two and one-half years duration later came to necropsy and the heart was regarded normal.

*The Effect of Alkali Administration on Gastric Acidity* By L C GATEWOOD, M D ,  
Chicago, Ill

In a group of patients undergoing treatment for gastric and duodenal ulcer, an opportunity arose to study the effect of prolonged alkali administration on the

secretion of hydrochloric acid. The diet was maintained practically constant and the patient was given doses of alkali just sufficient to neutralize the free hydrochloric acid. The quantity of alkali required, furnished, therefore, a measure of the amount of acid secreted. Curves are presented to show the variations in alkali required. In some instances there was a progressive increase in acidity to such high levels that it was not safe to control the acidity in this way. In some the level of acidity remained unchanged over long periods of time. In many the acid secretion decreased progressively to low levels or disappeared for variable periods of time. While the latter change was frequently coincident with the occurrence of alkalosis it has also occurred with no change in blood chlorides or CO<sub>2</sub> combining power of blood.

*Tuberculosis Among Nurses* By EVERETT K. GEER, M.D., Saint Paul, Minn.

From 1920 to 1928 forty two of nine hundred and thirty four nurses (4.5 per cent) at the Ancker Hospital in St. Paul developed tuberculosis. Because of this high incidence an investigation was instituted.

Beginning in September, 1928 all new nurses entering training were given intra-dermal tuberculin tests along with their customary physical examination and chest x rays. The new group in September 1928, showed that one third reacted positively to intradermal tuberculin. The rest of the training school was then tested and only four of one hundred forty seven were found negative.

Since then all nurses entering training have been tested and if negative re-tested every six months. This study has shown that in the past two years about 30 per cent of nurses entering the Ancker training school are positive and that by the end of their first year nearly all are positive.

The Ancker Hospital has a tuberculosis department of two hundred and fifteen beds and nurses have duty in this department before the end of their first year.

Nurses in other training schools in St. Paul were tested and average 42 per cent reactors in their third year.

Of one hundred and twenty two new nurses entering the Ancker training school in the past two years seven have developed tuberculous disease. Six of these were negative to 1 mgm. of old tuberculin on entering training. Three of the seven have developed pleurisy with effusion, two, parenchymatous lung lesions, one, a basal lesion of the childhood type and one erythema induratum with tracheobronchial adenopathy.

It is felt that by carrying out more rigid contagious technique in the tuberculosis department and by routinely taking a single chest film of all persons admitted to the hospital on all services that the incidence of tuberculous infection and disease among nurses can be materially reduced at Ancker Hospital and these procedures are to be instituted.

It is recommended that especially in those communities where the incidence of tuberculous infection is low among young adults that in tuberculous sanatoria and tuberculous departments of general hospitals, contagious technique should be employed. It is also considered advisable to routinely x-ray the chest of all

admissions to general hospitals to eliminate in part the unrecognized consumptive menace

*The Effect of Amyl Nitrite on Striated Muscle Spasms* By C D CHRISTIE, M D , Cleveland, Ohio

During the past year it has been found from extensive clinical trials that amyl nitrite when inhaled in sufficient dosage exerts a decided effect on the acute myalгias such as torticollis, lumbago, pleurodynia, etc In some instances the pain and spasm are entirely relieved, in others the effect, while beneficial, is transient There are some instances in which clinically the condition would seem to be an acute myalgia, but the inhalation of amyl nitrite gives no relief

Attempts have been made in the laboratory to produce a condition analogous to acute myalгias, in the muscles of animals, without success Attempts have also been made to counteract various kinds of experimentally increased muscle tone in animals, from the effect of caffeine, nicotine, cardisol, veratum, insulin, tetanus toxin, barium, guanidine, lactic acid, etc , without success, when amyl nitrite was used in a dosage at all comparable with that used in the clinical application of the drug

*Studies in Serum Viscosity in Hypertension* By EDMUND F FOLEY, M D , (by invitation), and C S WILLIAMSON, M.D , Chicago, Ill

Blood pressure is essentially the result of the force of the heart beat, the peripheral resistance, and the viscosity of the circulating fluid It is commonly appreciated that pathological conditions which increase the first two factors are frequently associated with hypertension This is a study of the third factor or the viscosity in cases of the hypertension of nephritis

In normal cases the viscosity as determined by Ostwald's method, temperature being controlled to 37°C was found to be from 1 794 to 2 07, average 1 86 In ten cases of severe nephritis, two of which were in uremia, and all showing marked evidences of renal decompensation, the maximum was 1 464, the minimum 1 351, the average 1 390 The two cases in uremia were less than the average being 1 365 and 1 351 This study would indicate that in nephritis hypertension is not due to increased viscosity of the serum

*A Comparison of the Heat Lost by Vaporization of Water Determined by the Basal Insensible Loss of Weight, with the Basal Heat Production of the Albino Rat* By JAMES A GREENE, M D , and P R LUCE, M D , (by invitation), Indianapolis, Ind Introduced by H W Gauchat, Canton, Ohio

The gaseous metabolism was determined by a method similar to Prince's The insensible loss of weight was obtained on a balance with a sensitivity of 0 5 mgm for a 300 gram load

The basal heat production compared favorably with that observed by others, and the percentage of heat lost by vaporization of water ranged between 20 63 and 26 80, with an average of 23 81 No difference was observed in the use of thin,

obese, and normal animals. The respiratory quotient was found to average 0.72, under standard conditions, and the insensible loss of weight was composed of 100 per cent of water with this respiratory quotient. Standard conditions were eighteen to twenty four hours without food or water at temperatures from 25 to 31°C.

*A Study of the Effect of Insulin on Gastric Motility* By T E HEINZ (by invitation), and W L PALMER, M.D., Chicago, Ill.

It is well known that insulin frequently produces an intense desire for food. Bulatao and Carlson have shown experimentally that it stimulates gastric hunger contractions. Hence it has been generally assumed that the hunger observed clinically after insulin administration is due to increased gastric motility.

In our experiments this has been tested and kymographic records of the gastric activity made. Definite insulin reactions were produced accompanied by a marked lowering of the blood sugar and frequently by a marked increase in the general desire for food.

The relationship between "hunger," "desire for food" and "hunger contractions" is discussed.

*Intestinal Hypersensitivity as an Etiological Factor in Angio-neurotic Edema* By STANLEY E DORST, M.D., Cincinnati, Ohio

Two patients with histories of well established angio-neurotic edema have been studied with the hope of establishing the existence of an allergic state. Foods, pollens, and the usual stock bacterial extracts were all employed with negative results. Various possible foci were then investigated, including the teeth, sinuses, nose, throat, and the enteric tract. All results were insignificant except those obtained using organisms recovered from the enteric tract. Marked reactions followed the intradermal injection of certain colonic strains. Typical attacks could be precipitated within a few minutes by a moderately large dose of the antigen. The patients were desensitized with these antigens with the results that one has gone seven months without an attack and the other fourteen months without symptoms.

*Unusual Phagocytosis by Reticular Cells in a Patient Having a Gram Negative Bacillus in the Blood* By FRANK J HECK, M.D., (by invitation), and H MILTON COVNER, M.D., Rochester, Minn.

The patient, a male, farmer aged forty five years, gave a history of ten months of fever, weakness, anemia, increased sense of warmth, sweating, and headache, and had ecchymotic areas on face for two years, with slight dyspnea on exertion. Examination showed a palpable spleen, skin ecchymoses, enlarged liver, positive tourniquet test and varying degrees of fever. Leukocyte count from ear varied between 35,000 and 78,000. In blood from finger and vein the count was never above (3,500). Morphologically there was unusual phagocytic activity by reticular

cells, with inclusion of whole polymorphonuclear neutrophiles, neutrophile remnants or erythrocytes. These reticular cells were seen only in blood from the ear and never in blood from the finger or vein. Six out of eight blood cultures on prolonged culture, two weeks or more, showed the presence of a hemophilic small Gram-negative bacillus of the influenza group.

*Treatment of Septic Meningitis by Intra-carotid and Cisterna Magna Injections*

By JAMES A EVANS, M D, LaCrosse, Wis

Review of literature and discussion of rationale of intra-carotid and intra-cisternal injections of pneumococcus anti-sera and pneumoquin-base for pneumococcus meningitis and anti-meningococcus serum for meningococcus meningitis.

Two cases of pneumococcus meningitis reported treated unsuccessfully by this method. Report of a very fulminating case of meningococcus meningitis treated unsuccessfully by this method.

*Lactic Acid in Human Stools* By J W PITTMAN, (by invitation), and W H OLMIESTED, M D, St Louis, Mo

The products of the action of *B. coli* on glucose in culture media are lactic, acetic and formic acids, alcohol, carbon dioxide and hydrogen. In previous work, the amounts of acetic acid found in stools under various conditions were determined and from this data the amounts of carbohydrate metabolized by bacteria were calculated. Since there are two molecules of lactic acid formed for one of acetic a corollary of our work would be the determination of the amounts of lactic acid found in stools.

*Method* The stools were mixed with a solution of 30 per cent mercuric sulphate dissolved in 10 per cent sulphuric acid. The mercury was precipitated by 10 per cent solution of sodium hydrate. After filtering, the traces of mercury were thrown out by hydrogen sulphide. To rid the filtrate of sugar, copper sulphate was added and precipitated with solid calcium hydrate. The final filtrate was water clear. Lactic acid in the filtrate was determined by the method of Friedemann Cotonio and Shaffer.

*Results* 1 In human adult stools there are at the most only traces of lactic acid.

2 Added lactic acid to stools may be recovered to the extent of 80 to 85 per cent. Recovery from watery solutions of lactic acid are 92 to 95 per cent.

3 If lactic acid be added to a stool and half of the stool sterilized and the other half incubated, lactic acid disappears from the incubated portion but can be recovered from the unincubated portion.

*Conclusions* 1 A rapid method for determination of lactic acid in stools is presented.

2 The bacterial flora of the intestinal tract in the adult individual rapidly destroys lactic acid.

*The Effect of the Combined Use of Ephedrine Hydrochloride and Digitalis or Ephedrine Hydrochloride and Ouabaine on the Unanesthetized Dog* By CARL A. JOHN SON, M.D., (by invitation), and N. C. GILBERT, M.D., Chicago, Ill.

By experiments upon unanesthetized dogs and by clinical observations the authors are of the opinion that the effect of the combined use of ephedrine and digitalis bodies in sub-lethal doses is distinctly toxic, especially in cases with small cardiac reserve.

The authors interpret their results as indicating that they are dealing with two cardiac poisons acting upon irritability and conductivity of cardiac tissue. They also feel that ephedrine in the doses given may produce some of the untoward effects observed from a secondary paralysis of the myoneural junction and the smooth musculature of the blood vessel as shown by Koppani and Luckhardt. This may increase the tendency toward a sudden fall in blood pressure.

In conclusion, they emphasize that when digitalis and ephedrine are used on the same patient, they should be given with extreme caution.

*The Occurrence of Digitalis in Ascitic and Edema Fluids of Patients Under Treatment with Digitalis* By GEORGE H. MILLER, M.D., Iowa City, Iowa

Using the Hatcher, cat assay type of experiment, the ascitic and subcutaneous fluids of certain patients under treatment for cardiac decompensation are shown to contain a digitalis-like substance. The amount of the drug present is sufficient to be of clinical significance if it were to enter the blood stream.

Hatcher has shown that digitalis is not eliminated to any great extent by the kidney. Since patients under effective treatment for cardiac decompensation will often put out enormous amounts of dropsical fluid by way of the kidneys, the possibility of digitalis poisoning from retention and concentration of digitalis must be considered.

We have seen clinical evidence suggestive of such a result when very rapid elimination of fluid was taking place.

*Spontaneous Peptic Ulcers of the Duodenum Following the Total Loss of Pancreatic Juice* By ROBERT ELMAN, M.D., St. Louis, Mo.

Peptic ulcers were found uniformly in dogs losing the total external secretion of the pancreas for two weeks or longer. The ulcers always developed just distal to the pylorus. The animals were all kept in good general condition and blood normal by intraperitoneal injections of Ringer's solution. Symptoms of gastric irritability were present in all cases. These observations fit in with a number of experiments by other workers and have a significant bearing on the problem of the pathogenesis and treatment of duodenal ulcer in man.

*A Note on the Prevention of Spinal Cord Degeneration in Pernicious Anemia* By PAUL STARR, M.D., Chicago, Ill.

A survey of the clinical course of eight of our patients having pernicious anemia and spinal cord degeneration indicates that they may be divided sharply into a

group of four in whom the neurologic symptoms have not progressed and a like number that have grown steadily worse. The first group has kept a count above five million throughout the period of three years' observation while those who have had increasing spinal cord difficulty have had more or less anemia during this period. There is a striking contrast between the average blood counts of these two groups. The anemic patients are not to be classed as liver failures since adequate liver has finally raised the blood count in each case. Neglect or gross infection produced the anemia.

*Conclusion* Those patients who received sufficient specific substance to keep the blood count above five million did not have progress of cord symptoms while those who were allowed to become anemic had progressive central nervous degeneration.

*Segmental Cutaneous Hyperalgesia as an Accurate Indicator of Visceral Disease*

By LEE D CADY, M D, St Louis, Mo

The utilization of the so-called Head zones of visceral pain reference has been almost totally neglected as a diagnostic test for visceral disease. Visceral organs that are inflamed or otherwise under tension usually cause characteristic patterns of hyperalgesia of the skin that are often of great value in differential diagnosis. The author's experience includes a study of these patterns in diseases of the aorta, heart, esophagus, gallbladder, liver, kidney, small intestine, appendix, colon, urinary vesical, prostate, salpinx and uterus.

*The Clinical Significance of the Electrocardiogram in Hypersensitive Heart Disease*

By THOMAS ZISKIN, M D, (by invitation) Introduced by Hugo Altnow, Minneapolis, Minn

Most observers believe that hypertension is an hereditary disease beginning in middle life, while some believe that it has its beginning in childhood and goes through various clinical stages. A comparative study was made of the clinical, x-ray and electrocardiographic findings in a series of 324 cases of hypertension.

It was found that the average period of onset of hypertension was in the fourth decade and that the condition runs a progressive course, reaching its maximum during the sixth decade. The size of the heart showed a corresponding progressive increase.

By means of the electrocardiogram, we can divide hypertensive heart disease into three stages: the irritative stage, the quiescent stage and the degenerative stage. The absence of the isoelectric phase in the S-T interval was characteristic of the irritative stage. The presence of the inversion of the T wave, arborization block and delayed conduction was characteristic of the degenerative stage. There was no definite relationship between the height of the blood pressure, the size of the heart and left ventricular preponderance. The presence of a depression or elevation of the S-T phase and other signs of myocardial degeneration was noted more frequently as the blood pressure increased.

The earlier in life a systolic blood pressure of 190 was reached, the more rapid was the course of the disease and the greater the damage to the heart muscle

*Serial Electrocardiographic Studies in Acute Coronary Thrombosis* By HUGO A FREUND, M D , and WARREN B COOKSEY, M D , (by invitation), Detroit, Mich

In the past two and one half years nineteen cases of coronary thrombosis were seen in private practice. By means of the portable electrocardiograph frequent tracings were made and in every one of the nineteen cases, evidence which we feel may be said to be diagnostic of coronary thrombosis was obtained. These findings are (1) Changes from the isoelectric level, of the S-T interval, or (2) development of the coronary T wave first described by Pardee, or (3) flattening or other changes in the T wave occurring in a short period of time, or (4) marked sudden fall in voltage of the Q R S. In six cases electrocardiographic tracings had been obtained previous to the infarction. Nine cases are dead and either the type of death or autopsy has verified the diagnosis. Lantern slides are to be shown summarizing the electrocardiographic changes in these cases, and a colored plate of a typical thrombosis with infarction.

*Blood Urobilin in Nitrogen Retention* By M A BLANKENHORN, M D , Cleveland, Ohio

Thirty patients with high blood urea were tested by estimating the blood urobilin according to a method previously described by the author. The average is compared with the average blood urea and uric acid. It appears that blood urobilin is "retained" when there is retention of nitrogen and to approximately the same degree.

*Thyrotoxicosis with a Normal Metabolism Early in the Course of the Disease* By FRED E BALL, M D , Chicago, Ill.

Occasionally patients are seen who present symptoms and physical findings of a thyrotoxicosis but who have normal metabolic rates. Three such cases are reported who later developed an increased metabolic rate within a period of a few weeks to two or three months. The symptoms did not change during that time. The suggestion is made that possibly some cases may be diagnosed as nervous when in reality they have a thyrotoxicosis.

*Hypometabolism in Youth and Young Adult Life* By JOHN TUCKER, M D , and E P McCULLAGH, M.D , (by invitation), Cleveland, Ohio

1 A study of hypometabolism in individuals between the ages of 15 and 30 years (inclusive)

2 Comparative study of two groups of these cases

(a) Those in which there is a slight lowering of the metabolic rate

(b) Those in which there is a marked lowering of the metabolic rate

3 Comparative study of these two groups of cases with relation to prominent symptoms

- (a) Those in which prominent symptoms of neurocirculatory asthenia are noted
- (b) Those in which the symptoms are more indicative of true hypothyroidism

4 A comparative study of the two groups of cases described above with regard to (1) symptoms, (b) physical signs, and (c) laboratory findings

5 A study of the response in cases in these groups to the administration of thyroid extract

*Hepatogenous Infection of Gallbladder* By EDMUND ANDREWS, M.D., Chicago, Ill

The rich bacterial flora of the normal liver, especially the presence of Welch bacilli. The increase in this flora and its spread to adjacent organs under experimental toxic conditions in the peritoneum. The sterility of normal dogs' bile. The prompt invasion of the gallbladder with microorganisms from the liver in cases of experimental cystic or common duct ligation. Evidences that this infection is hepatogenous because of the fact that the inflammatory reaction in the gallbladder is much more extensive on its hepatic surface and in early cases of cholecystitis is confined to the hepatic surface of the gallbladder.

## STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM

### VIII THE INFLUENCE OF THE THYROID GLAND AND THE PARATHYROID HORMONE UPON THE TOTAL ACID-BASE METABOLISM

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#### INTRODUCTION

The studies of Aub, Bauer, Heath, and Ropes (1) demonstrated that there is a marked increase in calcium excretion in hyperthyroidism and a decrease in calcium excretion in hypothyroidism. Moreover, these studies showed that the increased calcium in hyperthyroidism was partly in the feces, although predominantly in the urine. The present investigation was undertaken to determine if possible the wherefore of this increased calcium excretion. Three possible explanations had been suggested.

In the first place it seemed possible that there might be an associated hyperparathyroidism with hyperthyroidism and an associated hypoparathyroidism with hypothyroidism. That this is not the right explanation is suggested by the fact that in hyperthyroidism the serum calcium is only slightly elevated and there is an increased calcium excretion in the feces, whereas in hyperparathyroidism the serum calcium is much elevated and there is no increased excretion in the feces (see studies by Albright, Bauer, Ropes, and Aub (2)).

Secondly, the possibility occurred that the calcium of the bones in its capacity as a reserve supply of base might be excreted in hyperthyroidism to neutralize some acid. There are four known methods of neutralizing such acids:

I Titratable acidity, used particularly for the excretion of phosphates

- II Ammonia
- III Fixed Base from extracellular fluids (Gamble, Blackfan, and Hamilton (3))
- IV Calcium phosphate from the bones

These various mechanisms are to a certain extent simultaneously called into play. The fourth is often the least affected by acid diets (4). It is, therefore, fair to assume that if the marked increased excretion found in hyperthyroidism is due to an acidosis, there must also be obvious stimulation of the other three mechanisms.

It is true that the increased calcium excretion in both hyperthyroidism and in acidosis is associated with an essentially normal blood calcium level. On the other hand studies from this laboratory (4) show that the increased calcium excretion found in the feces in hyperthyroidism is not present in the disordered metabolism of acidosis.<sup>1</sup> This discrepancy seemed important, but perhaps not fundamental, for the increased fecal excretion in hyperthyroidism may merely be an expression of increased intestinal rate and increased excretion of gastrointestinal juices. It was thought, too, that the negative nitrogen balance, which was present in most of the cases of hyperthyroidism studied (1), might point to one source of acid. A negative nitrogen balance means that energy is being derived from the patient's own flesh,—really a meat diet, which, of course, is an acid diet because of its high sulphate and phosphate contents. This thought appeared to be supported by the observation that the negative nitrogen balances during fasting experiments were associated with calcium excretions in the urine almost comparable with those in hyperthyroidism (5, 6, 7). Furthermore, Benedict's fasting man showed a distinct parallelism between his negative nitrogen balance and his urinary calcium excretion. Of course, the large fecal calcium found in hyperthyroidism was not found in the starving man.

Finally, as a third possibility, it was thought that the thyroid hormone might have a direct stimulating catabolic effect on the calcium deposits in the bones. This, we appreciate, is no explanation of how

<sup>1</sup> Givens (8) and Givens and Mendel (9) likewise found no increase in fecal calcium in dogs as a result of acidosis although Stehle (10) found a very definite increase.

this catabolic effect is exerted It merely serves to differentiate it from other known methods of mobilizing bone salts

#### PLAN OF STUDY

In order to investigate the second possibility, namely, that the calcium excretion in hyperthyroidism might be increased because of a demand to help in the excretion of some acid—it was decided to do total acid-base studies By following the changes in acid and basic radicles from the hypothyroid state to the normal and from the hyperthyroid state to the normal we believed it would at once be apparent whether or not calcium was being called out to neutralize an excess of acid Furthermore, it was decided to study in a similar manner a normal subject before and during parathormone medication in order to see whether there was any analogy between the effect of the thyroid and parathyroid hormones on total acid base metabolism

In order to balance the basic factors in the urine against the acid factors, one has to know on the basic side

- (1) Fixed base excretion (= sum of Na, K, Ca, Mg)
- (2) NH<sub>4</sub> excretion
- (3) Titratable acidity (= base necessary to bring urine to a pH of 7.35)

and on the acid side

- (1) Chlorides
- (2) Sulphates
- (3) Base binding value of phosphates at pH 7.35
- (4) Undetermined acid (= mostly organic acids)
- (5) Carbonates

The sum of the acid factors expressed in cubic centimeters of N/10 should equal the sum of the basic factors expressed in cubic centimeters of N/10 (see Gamble (11)) Rather than undertake the necessarily difficult task of determining the carbonates, we have used a procedure described by Albright and Bauer (12) This consists in making both the cation and anion columns shorter by the carbonic acid value, i e on the cation side our third value is the "titratable acidity minus CO<sub>2</sub>" instead of titratable acidity and on the anion side we have not

Li

Period number	Calcium metabolism										Phosphorus metabolism (valence assumed at 1.8)										Nitrogen															
	Weight		Caloric intake		Fluid intake		Urine		Dried feces		Basal metabolic rate		Urine		Total output		Intake		Balance		Urine		Total output		Intake		Balance		Theoretical balance		Urine		Total output		Intake	
	kilos	calories	cc	cc	cc	cc	grams	grams	N/10	cc	N/10	cc	N/10	cc	N/10	cc	N/10	cc	N/10	cc	N/10	cc	N/10	cc	N/10	cc	N/10	cc	N/10	grams	grams	grams				
I	76.0	4096	7092	6590	67.2	-44	154	290	113	-177	-93	840	1197	948	-249	-81	21	10	23	48	23	-19	21	23	23	63	24	-19	21	23	23	63	24			
II	75.5	4095	7092	6290	41.5		98	172	113	-59	-31	530	715	948	233	19	21	23	23	63	24	-76	21	87	24	27	24	-76	21	87	24	27	24			
III	75.4	4143	7113	5370	51.8	-35	110	244	116	-129	-67	500	697	989	292	-76	21	87	24	27	24	-235	19	33	20	77	14	-235	19	33	20	77	14			
IV	74.3	3945	6257	5495	5.8	-46	90	105	67	-39	-20	470	558	552	-406	-235	19	33	20	77	14	-96	20	74	23	16	24	-96	20	74	23	16	24			
V	72.5	3999	7087	5905	88.8	-41	139	361	110	-251	-131	430	715	948	233	-96	20	74	23	16	24	-400	23	39	25	93	25	-400	23	39	25	93	25			
VI	73.1	4223	7113	5725	38.2	-42	224	303	116	-187	-98	110	1389	989	-400	-115	23	39	25	93	25	-483	34	25	36	76	25	-483	34	25	36	76	25			
VII	72.2	4229	7109	6260	31.7	-22	217	297	115	-182	-95	1230	1465	982	-483	-490	34	25	36	76	25	-605	-1	003	47	23	49	-605	-1	003	47	23	49			
VIII	71.4	4064	7047	5995	57.5		248	407	110	-297	-155	1180	1553	948	-605	-1	003	47	23	49	69	24	-1470	-1	037	48	45	50	-1470	-1	037	48	45	50		
IX	70.2	4309	7060	5580	70.1	-13	255	422	117	-305	-159	2160	2418	948	-1470	-1	037	48	45	50	95	25	-372	-1	316	56	68	59	-372	-1	316	56	68	59		
X	69.0	4281	7113	5545	40.7	-13	312	447	117	-331	-173	1180	1361	989	-372	-1	028	46	54	48	89	23	-714	-1	028	46	54	48	-714	-1	028	46	54	48		
XI	67.2	3596	6937	6455	31.8	-10	275	420	100	-320	-167	1360	1592	878	-714	-1	028	46	54	48	89	23	-856	-968	45	42	47	88	-856	-968	45	42	47	88		
XII	65.4	4247	7105	5665	44.9	-6	248	464	114	-350	-183	1500	1832	976	-856	-968	45	42	47	88	24	-581	-859	42	45	44	98	-581	-859	42	45	44	98			
XIII	65.4	4308	7113	5395	34.9	-1	320	494	119	-376	-196	1340	1570	989	-581	-859	42	45	44	98	25	-858	41	34	43	76	24	-858	41	34	43	76	24			
XIV	64.4	4039	7111	5340	35.5	+3	359	497	113	-385	-201	1380	1564	959	-605	-858	41	34	43	76	24	-635	-635	-635	-635	-635	-635	-635	-635	-635	-635	-635				
XV	63.2																																			
XVI	62.4	4223	7113	5120	53.4	+5	293	704	116	-588	-306	970	1273	989	-284	-527	29	55	32	09	23	-459	29	12	31	66	25	-459	29	12	31	66	25			

Total daily urine nitrogen balance	Total base metabolism						Sulphur		Chloride				Blood serum		Medication and treat ment per period
	Urine	Total output	Intake	Balance	Theoretical balance	Inorganic sulphur urine	Total sulphur intake	Urine	Intake	Ammonia	Titratable acidity - CO <sub>2</sub>	Organic acid	Calcium	Phosphorus	
N/10	cc. N/10	cc. N/10	cc. N/10	cc. N/10	cc. N/10	cc. N/10	cc. N/10	cc. N/10	cc. N/10	cc. N/10	cc. N/10	cc. N/10	mg. 100 cc.	mg. 100 cc.	
13	3 487	4 337	4 098	-239	-164	519	1 185	2 160	1 971	793	433	1 194	9 6	3 5	
15	3 144	3 779	4 098	319	-44	586	1 185	1 963	1 971	765	303	1 138			
-11	3 713	4 553	4 182	-371	-140	695	1 272	2 586	1 977	785	324	1 041			
-260	3 114	3 759	2 753	-1 006	-299	530	909	1 972	1 597	601	173	919	10 4	3 9	
42	3 554	4 764	3 757	-1 007	-209	643	1 206	1 470	1 697	849	212	2 072			0.2 gm. thyroid extract
-22	3 039	3 743	4 182	439	-209	618	1 272	1 763	1 977	1 051	443	1 042			1.2 gm. thyroid extract
-476	2 980	3 719	4 153	434	-658	1 082	1 263	1 740	1 955	1 338	675	941			1.2 gm. thyroid extract
-1 024	3 000	4 390	4 023	-367	-1 321	1 569	1 254	1 457	1 964	1 411	976	1 181			1.2 gm. thyroid extract
-1 059	3 495	5 095	4 128	-967	-1 364	1 676	1 241	1 590	1 974	1 371	966	406			1.2 gm. thyroid extract
-1 380	4 644	5 654	4 186	-1 478	-1 711	906	1 272	2 762	1 977	1 161	962	919	10 6	5 5	1.5 gm. thyroid extract
-1 040	4 000	4 790	3 581	-1 209	-1 360	1 661	1 136	1 916	1 630	949	840	852			1.7 gm. thyroid extract
-950	3 530	4 605	4 154	-451	-1 300	1 559	1 243	1 455	1 974	1 041	964	1 021			1.9 gm. thyroid extract
-803	3 183	4 193	4 180	-13	-1 179	1 477	1 267	1 570	1 975	904	915	615			2.1 gm. thyroid extract
-798	2 999	3 869	3 886	17	-1 183	1 410	1 217	1 618	1 717	1 062	834	487			1.3 gm. thyroid extract
															Sent home for two weeks on normal diet but continued thyroid therapy
-270	3 120	4 259	4 185	-74	-858	946	1 272	1 802	1 977	731	637	779	10 6	5 5	0.9 gm. thyroid extract
-255	3 456	4 446	4 185	-261	-736	893	1 272	2 020	1 977	734	670	917			0.9 gm. thyroid extract

determined the carbonic acid. One obtains the "titratable acidity minus CO<sub>2</sub>" value by adding a known amount of mineral acid to the urine, blowing off the carbon dioxide, titrating with standard alkali back to a pH of 7.35, and then subtracting the acid added from the alkali used. The "undetermined acid" was not measured but taken as the difference between the sum of the basic radicles and the sum of the other acid radicles. During the remainder of the paper this value will be called "organic acid." It must be remembered that, derived as it is, the organic acid value contains all the errors of the experiment.

The three patients studied were carefully chosen. The cases of myxedema and exophthalmic goiter were classical examples of the severest types, and during our metabolic study both women essentially recovered. The patient to whom parathormone was given was a normal man except for a calcified hematoma in his thigh. The diets used were nearly neutral, low in calcium, and satisfied the desires of the patient although necessarily not the caloric requirement. The diet as well as the water intake was kept constant throughout each study. The variations which occurred in their metabolism, therefore, were dependent upon changes in the patients' conditions and not in their diets. The patients were, therefore, their own controls. The periods of study were three days each. The method of collecting urine and feces was that used in other studies (13). The chemical methods used were as follows: for calcium, McCrudden (25), for phosphate, Fiske and Subbarow (26), for ammonia, Folin (27), for titratable acidity, Folin (28), for total base, Fiske (29), for sulphur, Fiske (30), for chloride, Van Slyke (31), for nitrogen, the Kjeldahl method, and for serum calcium, Clark and Collip (32).

## RESULTS

### PART I

#### *Effect of thyroid extract on patient with myxedema*

(The data for Part I is given in table 1 and chart 1) Mrs L C had severe myxedema. She was a very cooperative patient, who was maintained on a constant diet and fluid intake throughout this observation although in several periods (IV, V, and XI) her dietary intake

was not quite so constant as in other periods. During the first five periods (i.e., 15 days) she received no thyroid medication, so that this period represents the metabolism of complete myxedema. Throughout the rest of the observation she received Armour's thyroid extract daily by mouth. Periods VI to IX inclusive represent the marked change of beginning of thyroid medication (0.4 gram per day), and the next five periods (X to XIV inclusive) represent thyroid medication after equilibrium has been more nearly established. In order to be sure that

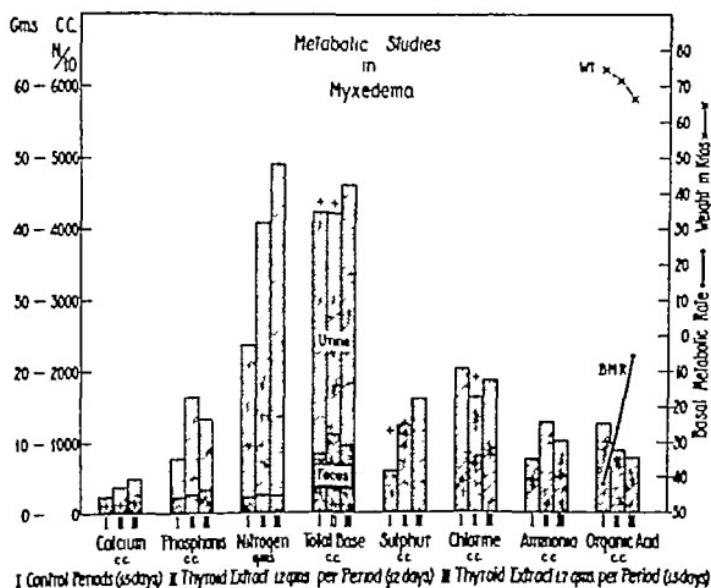


CHART 1

this represented the permanent stage metabolically, the patient was sent home for two weeks on constant thyroid medication but without dietary restrictions. She then returned to the metabolism ward on the previous regime and periods XV and XVI were obtained, which represent the steady state of her normal metabolism. Thus the entire investigative period can be divided into four main subdivisions:

- (a) Periods I—V, control periods before thyroid medication
- (b) Periods VI-IX, showing marked changes at beginning of thyroid medication

- (c) Periods X-XIV, after equilibrium has been more nearly established, and
- (d) Periods, XV-XVI, showing established thyroid medication

#### *Water balance*

It is a striking fact that in spite of a constant fluid intake, but a marked loss in weight, the quantity of urine excreted per period was practically uninfluenced by thyroid feeding. Any increased fluid, therefore, was eliminated either through feces or through insensible perspiration, values which were not established in our observations.

#### *Protein metabolism*

Thyroid extract produced a marked effect upon the protein metabolism in this patient, for the nitrogen equilibrium, established during the control period, changed to a very marked negative nitrogen balance (equivalent to 24.4 grams N per period) during periods X-XIV. There was still an average negative nitrogen balance of 6.5 grams during periods XV and XVI after three weeks of continued thyroid medication.

#### *Calcium metabolism*

The conclusions in Paper III (1), in regard to calcium excretion in myxedema, are confirmed by this patient. The calcium elimination before treatment was very much below normal, but after thyroid was taken the calcium excretion more than doubled in the urine and it also rose in the feces. As pointed out in a previous publication (14), this increase in calcium excretion in the feces is a unique effect of thyroid, as other methods of increasing calcium excretion affect urinary excretion alone. This loss of calcium is quite out of keeping with the loss in body weight as in these 5 periods (X-XIV) the loss of body weight was only 2.6 kgm more than in the control five periods. If one figured that the total loss of weight was due to body fluids and that these fluids contained 10 mgm of calcium per 100 cc as in serum, then the calcium excretion should have increased 260 mgm. The actual increased calcium output (2300 mgm) would still be far in excess of this calculation. We are, therefore, again forced to the conclusion that the excess calcium was derived from the bones. It is clear that this

urinary calcium is markedly increased during the first three days of thyroid medication. This increase is also obvious in the phosphorus excretion, but the nitrogen elimination and the basal metabolic rate are not increased until the second period of thyroid feeding. It is thus evident that the response in the calcium metabolism to thyroid medication is partially independent of these other factors. This is confirmed in our studies of the treatment of parathyroid tetany with thyroid medication (Paper VI of this series (15)).

TABLE 2

*The effect of thyroid administration on calcium excretion in the case of myxedema*

Periods	Urine excretion	Total excretion	Balance	Remarks
	$\text{cc N/10}$	$\text{cc N/10}$	$\text{cc V/10}$	
I-V	118*	234	-131	Before thyroid treatment
VI-IX	236	357	-243	First 4 periods of thyroid treatment
X-XIV	303	464	-352	5th-9th periods of thyroid treatment
VV-VVI	306	650	-534	Periods of established thyroid medication

\* The average value per period is given in each case.

### *Phosphorus metabolism*

In table 3 are given the data for the phosphorus balances. By "theoretical balance" as described in an earlier paper (2) we mean that part of the phosphorus balance which is explainable by the calcium balance and by the nitrogen balance. This is based on the assumption that any balance of calcium represents  $\text{Ca}_3(\text{PO}_4)_2$  in bones ( $\text{Ca:P} = 1.9$ )<sup>2</sup> and that any balance of nitrogen represents protein ( $\text{N:P} = 17.4$ ). When the actual balance is less than the theoretical balance it should point to a loss of phosphorus from body fluids.

In table 3 one notes a tremendous increase in phosphorus excretion during thyroid medication. This was to be expected because of the large negative nitrogen balance. During the control periods (I-V) and

<sup>2</sup> Calcium has been thought to be deposited largely as tertiary calcium phosphate ( $\text{Ca:P} = 1.93$ ) but is also partly deposited as calcium carbonate so that the ratio of calcium to phosphorus in bone is approximately 2.23.1. For relatively rough calculations like the present it makes little difference whether one uses the factor 1.9 or 2.2. In this paper we have arbitrarily chosen 1.92.

the periods of established thyroid feeding (XV-XVI), one notes that not so much phosphorus was excreted as one would expect from the theoretical balance. This probably means that the phosphorus calculation from the diet was slightly too high. The interesting thing is that during the transition periods (VI-IX) more phosphorus was excreted than one should have expected, in contrast to these other periods. This occurred in spite of a rising blood phosphorus (see table 1). Again, even if the 4.1 kgm loss of body weight during this period were entirely body fluids, which of course it was not as shown by the negative nitrogen balance, and if this body fluid were thought of as containing 4 mgm of phosphorus per 100 cc, only 164 mgm of phosphorus would be accounted for. It would appear that the imme-

TABLE 3  
*The effect of thyroid administration on phosphorus excretion in the case of myxedema*

Period	Intake cc N/10*	Output cc N/10	Actual balance cc N/10	Theoretical balance cc N/10	Excess of actual over "theoretical balance" cc N/10
I-V	877†	776	+101	-101	+202
VI-IX	967	1706	-739	-661	-78
X-XIV	958	1584	-626	-1006	+380
XV-XVI	989	1325	-336	-493	+157

\* Phosphate figures are all reduced to cc N/10 base which would be bound by phosphate at a pH of 7.35.

† The average value per period is given in each case.

diate effect of the thyroid hormone is to excrete phosphorus in excess of what can be explained by the increased nitrogen and calcium excretions. Furthermore, by comparing periods X-XIV with periods VI-IX, one gets the impression that the increased excretion of phosphorus at first is later compensated for. Thus if one averages periods VI-XIV together one obtains an average excess of actual over theoretical balance per period of +176, which is not far from that noted in the control periods. It would seem that the thyroid hormone had caused a breakdown of protein with an immediate excretion of the phosphorus and a later excretion of the nitrogen. Boothby, Sandiford, Sandiford and Slosse (16) found a temporary increase in the nonprotein nitrogen of the blood of two myxedema patients under thyroid treatment.

It should be further noted that the increased phosphorus excretion is entirely in the urine

#### *Acid radicles in urine*

We have now to examine the acid elements which were excreted in the urine to see whether they increased appreciably under thyroid medication (see table 4) These factors are phosphates, sulphates, chlorides, and organic acid radicles

The *phosphates* have been discussed above

As was to be expected from the increased nitrogen excretion under thyroid medication, there was a corresponding increased *sulphur* excretion The ratio of the increase in nitrogen excretion to the

TABLE 4

*The effect of thyroid administration on acid excretion in the urine in the case of myxedema*

Periods	Phosphates	Inorganic sulphates	Chlorides	Organic acid radicles	Total acids minus carbonates
I-V	cc N/10*	cc V/10	cc N/10	cc V/10	cc N/10
VI-IX	554†	595	2 030	1,273	4,452
X-XIV	1 420	1 236	1,638	892	5,186
XV-XVI	1 352	1 603	1 864	779	5,598
XV-XVI	1 000	919	1,911	848	4 678

\* Phosphate figures in terms of cc. N/10 base which would be bound by phosphates at a pH of 7.35

† The average value per period is given in each case

increase in inorganic sulphate excretion was during periods VI-IX, 17.3, during periods X-XIV, 15.8, and during periods XV-XVI, 17.3 This corresponds very closely with the ratio of 17.5 found by Gamble, Ross, and Tisdall (17) in fasting children We may, therefore, assume that the increased sulphate excretion represents the oxidized sulphur from the increased protein catabolism

It is interesting that during the whole observation of 48 days 416 cc N/10 more chloride was eaten than was excreted in the urine This is a variation of only 1.3 per cent of the intake It is difficult to understand why more chloride was not excreted as a result of the large loss of weight It is of special interest that least chloride was excreted during periods VI-IX and periods X-XIV when the loss of weight and

nitrogen were at their height. One wonders whether the chloride excretion in the urine was not diminished in these periods as a compensation to the increased sulphate and phosphate excretion. We did not examine the chloride excretion in the feces for it has not been considered a large factor. It is possible, however, that the elimination of chloride here may have been appreciable and possibly more so at the time when other acids were increased in the urine.

Like the chloride excretion, the *organic acid* excretion was diminished during the periods of increased sulphate and phosphate excretion. This is in agreement with the observation made by Albright and Bauer (12) from a similar type of experiment that organic acid excretion is diminished in the urine during periods of increased excretion of acid radicles and increased during periods of increased excretion of basic radicles. It would appear that organic acid behaves somewhat like carbonic acid in this respect (see Gamble (11)).

In the last column of table 4 are given the figures for the *total acid excretion* in the urine. One notes that the net result is a gain of about 1000 cc of N/10 per three-day periods during the first 27 days (periods VI-XIV) of thyroid medication. During the final periods (XV-XVI) the acid excretion has returned to approximately that found before thyroid medication. It would appear that the increased sulphate and phosphate excretions, resulting largely from the negative nitrogen balance, are not entirely compensated for by decreased chloride and organic acid excretion and that thyroid medication in this patient at least temporarily increased the sum total of acid electrolytes appearing for excretion in the urine.

#### *Basic radicles in urine*

We must now examine the basic radicles in the urine to see by what measures the increased acid excretion was met (see table 5). The basic factors are titratable acidity minus CO<sub>2</sub>, NH<sub>4</sub> excretion, and total fixed base excretion.

The *titratable acidity minus CO<sub>2</sub>* showed the expected increase during the periods of increased acid excretion.

Likewise, the *ammonia* excretion was increased as a result of thyroid medication. Thus, two lines of defence against an acidosis had been partially mobilized.

When one examines the *total base* excretion (see table 1<sup>3</sup> as well as table 5), one is surprised to find that it is very little affected by thyroid medication. What little increased excretion there is, mostly in the feces, can be accounted for by the calcium lost from the bones rather than by base held in body fluids. As with the chloride excretion, one does not find even the increase which one would expect from the loss of weight and nitrogen (see calculation in footnote and table 1). Following the conception of Boothby, Sandiford, Sandiford and Slosse (16) we are led to believe that the loss of nitrogen represents deposit protein rather than true protoplasm and that this deposit protein does not hold water and salts to the same extent as structural protoplasm.

TABLE 5

*The effect of thyroid administration on excretion of basic radicles in the urine in the case of myxedema*

Periods	Titratable acidity minus CO <sub>2</sub>	NH <sub>4</sub>	Fixed base	Sum of basic factors
	cc. N/10	cc. N/10	cc. N/10	cc. N/10
I-V	290	759	3 403	4 452
VI-IX	765	1,293	3,128	5,186
X-XIV	903	1 024	3,671	5,598
XV-XVI	653	732	3 293	4 678

\* The meaning of "titratable acidity minus CO<sub>2</sub>" has been discussed in the text.

Finally, when we examine the basic factors as a whole in the urine we find that the increased acid excretion is adequately taken care of

<sup>3</sup> By "theoretical total base balance" in table 1 is meant the total base balance which one would expect from the calcium balance and the nitrogen balance. Thus, if 100 cc. of N/10 calcium were lost from the bones one would expect the total base balance to be minus 100, other things being equal. Likewise, if nitrogen were lost from the body, one would expect the base held by the muscle water thus liberated to be excreted. In calculating the amount of muscle water liberated from the nitrogen balance, the following formula taken from Gamble, Ross, and Tisdall (17) has been used:

$$N \times 29.5 \times 0.76 = \text{muscle water}$$

The first factor provides an estimate of the protoplasm destroyed and the second indicates the corresponding water content. The total base content of muscle water has been taken to be 180 cc. of N/10 per 100 cc. (17)

by an increase in the acidity of the urine and by an increase in ammonia excretion from body fluids, and that, consequently, the increased calcium excretion, the "fourth line of defence," is probably not acting in its capacity as a reserve supply of base but is caused by some other factor. This reasoning gains support by a comparison of the figures in this case with those obtained by administration of ammonium chloride to a patient with nephrosis studied by Albright and Bauer (12), and by the administration of phosphates and chlorides studied by Farquharson, Salter, Tibbetts, and Aub (4). These patients responded to acid ingestion by marked increase in total base excretion.

#### SUMMARY

The feeding of thyroid in this case of myxedema produced the following effects. The basal metabolism rose from minus 42 per cent to plus 5 per cent during the observation. With this change in the metabolic rate there was a loss of weight with a marked negative nitrogen balance. As a result of the negative nitrogen balance there was a large increase in the excretion of sulphates and phosphates in the urine. This increased acid value of the urine was somewhat diminished by a decreased excretion (? compensatory) of chlorides and organic acid radicles in the urine. The net increased acid value of the urine was entirely met on the basic side by an increase in titratable acidity and  $\text{NH}_4^+$  excretion, so that there was no increased excretion of total base in the urine, other than the slight increase that could be accounted for by the increased calcium excretion. It is, therefore, thought unlikely that the increased calcium excretion which occurred in both urine and feces was due to a demand for base to help in the excretion of acid.

The figures also showed that the increased phosphorus excretion was at first more than could be explained by the increased calcium and nitrogen excretion, but that there was apparently later a compensatory retention of phosphorus.

From the fact that the increased nitrogen excretion was not associated with an increased excretion of total base and chloride it is believed that this nitrogen might represent destruction of deposit protein rather than of structural protoplasm and that deposit protein has a different relation to intra-cellular water and salts than has structural protein.

## PART II

*Effect of Lugol's solution and subtotal thyroidectomy on a patient with exophthalmic goiter*

(The data for Part II is given in chart 2 and table 6) The patient, Emma F, was a young woman who was suffering from a very acute and severe exophthalmic goiter. The six weeks' study includes the

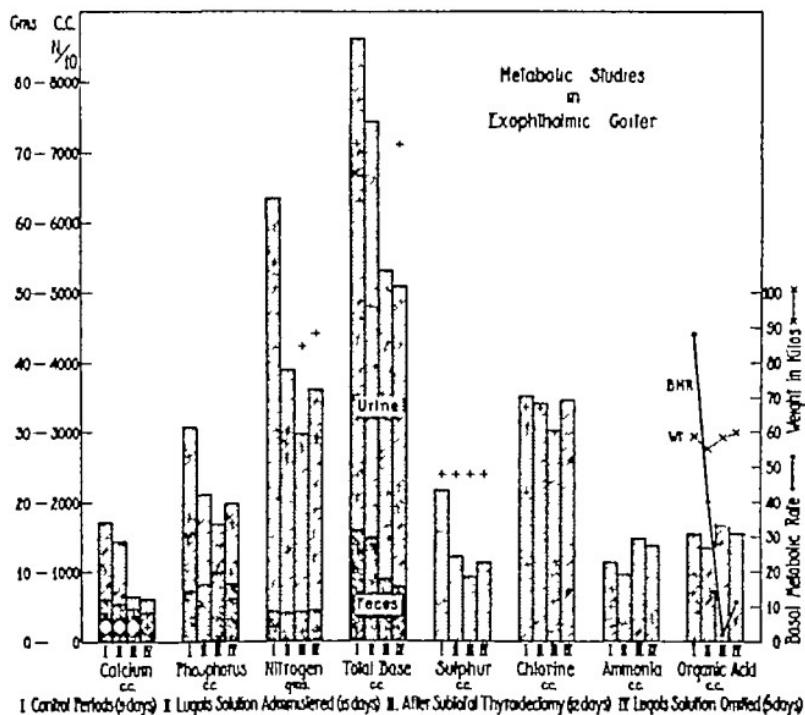


CHART 2

whole period of dramatic change from the severest type of the disease back to normal. Her basal metabolism changed from plus 99 per cent to a normal figure. The entire study of 16 three-day periods may be divided into

- (a) Control periods before treatment (periods I-III)
- (b) Periods with Lugol's medication (periods IV-VIII)

Emma F, aged 28 Admitted April 14, 1927 Discharged June 5, 1927 Diagnosis Exophthalmic

Period number							Calcium metabolism						Phosphorus metabolism (valence assumed at 8)						Nitrogen																	
	Weight		Caloric intake		Fluid intake		Urine		Dried feces		Basal metabolic rate		Urine		Total output		Intake		Balance		Phosphorus equivalent of calcium balance		Urine		Total output		Intake		Theoretical balance		Urine		Total output		Intake	
	<i>kglos</i>	<i>calories</i>	<i>cc</i>	<i>cc</i>	<i>grams</i>	<i>cc</i>	<i>N/10</i>	<i>cc</i>	<i>N/10</i>	<i>cc</i>	<i>N/10</i>	<i>cc</i>	<i>N/10</i>	<i>cc</i>	<i>N/10</i>	<i>cc</i>	<i>N/10</i>	<i>cc</i>	<i>N/10</i>	<i>cc</i>	<i>N/10</i>	<i>cc</i>	<i>N/10</i>	<i>cc</i>	<i>N/10</i>	<i>cc</i>	<i>N/10</i>	<i>cc</i>	<i>N/10</i>	<i>cc</i>	<i>N/10</i>	<i>cc</i>	<i>N/10</i>			
I	59.2	8997	11802	5460	93.4	+99	700	1368	216	-1152	-602	2182	2915	1700	-1215	-1220	5819	6260	44																	
II	58.6	8938	11787	4605	89.1	+95	1176	1738	215	-1528	-797	2498	3230	1674	-1560	-1648	6421	6851	43																	
III	57.8	8934	11760	6320	71.5	+69	1425	2016	214	-1801	-940	2327	3055	1577	-1478	-1673	5491	5913	42																	
IV	57.0	13600	5745	739			1161	1685	207	-1478	-771	1826	2428	1373	-1055		3848																			
V	59.0	0033	13600	7550	69.6	+47	1276	1943	216	-1727	-900	1162	1965	1700	-265	-816	3719	4160	41																	
VI	58.0	0028	13600	8275	78.4		887	1320	216	-1104	-576	1092	1758	1699	-59	-263	3033	3474	41																	
VII	57.7	9157	13600	6770	70.7	+34	663	1284	216	-1068	-557	1144	1952	1700	-252	-304	3211	3652	41																	
VIII	57.2	6102	13600	5510	71.1		772	1495	216	-1279	-666	1206	2425	1700	-725	-367	3615	4056	41																	
IX	53.0	006	11760	5365			662		193			1256		339				3672	3856	11																
X	57.0	7560	13005	8180	41.5		209	673	192	-482	-252	522	1299	1170	-129	2725	0328	74	3																	
XI	57.6	9033	13600	9185	59.4	+11	116	650	216	-434	-226	389	1358	1701	343	27924	5628	97	41																	
XII	58.5	9033	13600	9020	76.8	-7	156	713	216	-497	-259	598	2389	1700	-689	21525	4929	90	41																	
XIII	59.4	9147	13600	9140	72.0		210	561	216	-345	-180	681	1220	1700	480	26226	4030	80	41																	
XIV	59.4	9153	13600	9505	66.5		190	555	216	-339	-177	1276	2125	1699	-426	22727	6032	00	41																	
XV	59.5	9153	13600	10310		+5	210	643	216	-427	-222	1496	2430	1700	-730	-1233	4037	80	41																	
XVI	60.2	9153	13600	9085	72.5	+16	195	652	216	-436	-227	1206	2178	1700	-478	-366	3400	3840	41																	

\* Constant low calcium diet. Protein 1.5 gram per kilogram per day    Calories 45 grams per kilogram per day    Fluid 3000 cc.

URINE cc. 10	Total base metabolism				Sulphur		Chloride				Blood serum		Medication and treatment per period	
	Total output cc. N/10	Intake cc. N/10	Balance cc. N/10	Theoretical balance cc. N/10	Inorganic sulphur urine cc. N/10	Total sulphur intake cc. N/10	Urine cc. N/10	Intake cc. N/10	Amonium cc. N/10	Titratable acidity - CO <sub>2</sub> cc. N/10	Organic acid cc. N/10	Calcium m.m. per 100 cc.	Phosphorus m.m. per 100 cc.	
507.7 927	7 104	-823	-1 900	2 100	2 505	2 974	3 388	1 258	1 264	1 573				
310.8 570	7 104	-1 460	-2 560	2 378	2 523	3 427	3 255	1 170	1 616	1 493	11.5	5.5		
730	7 104	-2 485	2 010	2 465	4 168	3 377	975	1 339	1 544					
045.7 343	6 279	-1 064	1 463	2 790							11.8	3.5	Lugol's solution 45	
200.8 041	7 100	-941	-1 626	1 313	2 474	4 110	3 388	1 119	620	1 355	11.8	4.1	drops per day	
590.7 806	7 100	-706	-726	1 131	2 560	4 435	3 384	1 040	496	1 371	11.1	4.3	Fluid intake increased to 3600 cc. daily	
620.7 311	7 100	-211	-762	1 210	2 560	3 472	3 388	1 061	636	1 510				
200.7 420	7 100	-320	-1 136	1 407	2 560	3 357	3 345	958	449	637	10.7	3.9		
023				1 509	1 219	3 066	1 266	2 453	717				Subtotal thyroidectomy	
350.3 473	7 100	3 627	-145	1 066	1 861	2 552	3 222	1 904	190	996				
560.3 784	7 100	1 316	177	805	2 538	3 446	3 388	1 482	165	1 567				
200.6 016	7 100	1 084	67	986	2 538	2 784	3 388	1 188	238	2 258	9.2	5.9		
870.5 989	7 100	1 111	189	824	2 538	3 294	3 388	1 322	450	1 846	9.6	4.1	Lugol's solution discontinued	
500.6 383	7 100	717	150	922	2 538	3 730	3 388	1 308	804	1 777				
200	7 100		-173	1 264	2 538	3 003	3 388	1 426	859	1 722				
160	7 100		-206	1 216	2 538	3 642	3 388	1 392	1 015	800	10.2	4.0		

- (c) Periods directly following subtotal thyroidectomy in which Lugol's medication was continued (periods IX-XII)
- (d) Periods following cessation of Lugol's medication (periods XIII-XVI)

During the entire study she had almost the same food, water, and salt intake daily except for the period which included her operation

#### *Water balance*

With the decreasing metabolism resulting from treatment there was an increased urinary excretion of water, in spite of a gain in weight. This diuresis, furthermore, was associated with a diminished excretion of urinary constituents. It is probable that this increase in urine was due to the decrease in the insensible perspiration associated with the decrease in basal metabolism.

#### *Nitrogen metabolism*

The urinary nitrogen excretion was very high at first, resulting in a considerable negative nitrogen balance during the first three control periods, but fell very rapidly under treatment so that a positive nitrogen balance was obtained during period V and thereafter with the exception of the time of the operation. The assumption is made that the fecal nitrogen is 10 per cent of the intake.

#### *Calcium metabolism*

In this patient, a marked increased calcium excretion in the urine and feces was found as in the cases of exophthalmic goiter which have already been reported. The urinary calcium excretion during the three control periods was 6.5 times normal and the fecal calcium excretion 2 times normal. By "normal" we mean the calcium excretions of a series of normal men under a similar régime (18). It is most significant that this increased calcium excretion, although eventually brought almost to normal by treatment, remained nevertheless excessive after nitrogen equilibrium had been established (see table 7). This in itself is strong evidence that if the increased calcium excretion in hyperthyroidism is due to an acidosis, it is not simply due to the acidosis resulting from burning protein. Further evidence for this

will be found in Paper XIII (4) of this series a normal man (R F F) on a high protein diet excreted 83 grams of urinary nitrogen in three days. While this indicates a far higher protein catabolism, his calcium excretion remained less than one fourth that found at first in this case of exophthalmic goiter.

TABLE 7

*The effect of treatment on calcium excretion in the case of exophthalmic goiter*

Periods	Urine excretion	Total excretion	Balance	Remarks
	cc N/10	cc N/10	cc N/10	
I-III	1 100*	1,707	-1 494	Before treatment
IV-VIII	952	1 545	-1 331	Lugol's medication
IX-XII†	286	679	-471	Following subtotal thyroidectomy with continuation of Lugol's solution
XIII-XVI	201	603	-387	Following omission of Lugol's solution

\* The average value per period is given in each case.

† In obtaining the averages the operative period (period IX) is omitted.

TABLE 8

*The effect of treatment on phosphorus metabolism in the case of exophthalmic goiter*

Periods	Intake	Output	Actual balance	"Theoretical balance"	Excess of actual over "theoretical balance"
	cc N/10	cc N/10	cc N/10	cc N/10	cc N/10
I-III	1 650†	3 067	-1 417	-1,514	+97
IV-VIII	1 634	2 105	-472	-438	-34
IX-XII‡	1 524	1,682	-158	+174	-332
XIII-XVI	1 700	1,988	-288	+28	-316

\* Phosphate figures are all reduced to cc. N/10 base which would be bound by phosphate at a pH of 7.35

† The average value per period is given in each case

‡ In obtaining the averages, the operative period (period IX) is omitted

### *Phosphorus metabolism*

Table 8 shows an analysis of the phosphorus data. With the decrease in the negative nitrogen balance with treatment there was a marked reduction in the phosphorus excretion. Later there was a further reduction corresponding to the decreased calcium excretion. By a comparison of the actual and theoretical phosphorus balances it

will be seen that there was very close agreement at first. This supports the observation that the phosphorus lost from the body in hyperthyroidism is only what would be expected from the calcium and nitrogen losses (1). During recovery a little more phosphorus was excreted than could be accounted for by the calcium and nitrogen balances. As in the case of myxedema under treatment the phosphorus changes were almost entirely in the urine.

#### *Acid radicles in urine*

Table 9 has been constructed similar to table 4 to show the effect of treatment on the acid radicles in the urine.

TABLE 9

*The effect of treatment on excretion of acid radicles in urine in the case of exophthalmic goiter*

Periods	Phosphates	Sulphates	Chlorides	Organic acid radicles	Total acids minus carbonates
I-III	cc N/10*	cc N/10	cc N/10	cc N/10	cc N/10
IV-VIII	2,336†	2,163	3,523	1,538	9,560
IX-XII‡	1,286	1,305	3,633	1,373	7,597
XIII-XVI	503	952	2,928	1,607	5,990
	1,165	1,056	3,417	1,536	7,174

\* Phosphate figures in terms of cc N/10 base which would be bound by phosphate at pH of 7.35.

† Average value per period is given in each case.

‡ In obtaining the averages, the operative period (period IX) is omitted.

The phosphate excretion has been discussed above and shows the marked fall under treatment with the terminal rise on the omission of Lugol's solution, thus paralleling the basal metabolic rate and the nitrogen excretion.

The sulphate excretion likewise parallels the nitrogen excretion. The ratio of urinary nitrogen excretion to the urinary inorganic sulphate excretion remains at about the level of 17.5 which Gamble, Ross, and Tisdall (17) found in children during fasting 16.9 in periods I-III, 16.7 during periods IV-VIII, 17.2 during periods X-XII, and 17.4 during periods XIII-XVI.

The intake and output of chlorides over the whole period of observation are remarkably close. During this study of 48 days she consumed

550 cc. more N/10 chloride than were found in the urine. This is approximately one per cent of the total intake and is a very close approximation of the theoretical amount she should have retained considering the total gain of 1 kgm in weight. Of course, during the period when she was losing weight her chloride excretion was higher than when she was depositing tissue. Thus, the low excretion of chlorides during periods IX-XII can be accounted for by the rapid gain in weight here. It appears, therefore, that the tissue losses and gains in exophthalmic goiter have essentially a normal chloride content in contradistinction to that found in myxedema.

The excretion of *organic acid* radicles remains on the whole quite constant although here again it tends to be highest when the other acids are lowest. Considering the large metabolic changes which occurred during this observation, the organic acid figures are remarkably constant.

When the figures giving the *total acid* excretion are compared it will be noted that there was a marked decrease in acid excretion as a result of treatment and that the total acid excretion followed the basal metabolism in a quite parallel curve. Except for the difference in the behavior of the chlorides it may be said that the acid radicle individually and collectively behaved oppositely to what they did in the case of myxedema under treatment.

#### *Basic radicles in urine*

Table 10 shows how the fluctuations in the acid excretion were met. The figures for the "*titratable acidity minus CO<sub>2</sub>*" show the expected parallelism with the total acid excretion. Since the titratable acidity value of the urine is largely dependent on the buffer action of the phosphates one would expect it to be increased when a large amount of phosphate was being excreted.

The *ammonia excretion* is very surprising. It shows no parallelism with the acid excretion and is actually highest when least acid is present for excretion. This is all the more surprising when one realizes that the total nitrogen elimination was lowest when the ammonia excretion was highest. The ammonia mechanism is not called into use in this case of hyperthyroidism and the increased acid excretion is apparently completely taken care of by other factors. In other ex-



and that the ammonia mechanism was never needed to help in the excretion of acid. Furthermore, the increased total base excretion during the hyperthyroid state was probably to be explained by the increased calcium excretion and the increase of base from intracellular fluid resulting from destruction of protoplasm, so that there was really no evidence of the "third line of defence" acting, namely, the excretion of base from extracellular fluid supplies.

#### SUMMARY

A patient with severe exophthalmic goiter (basal metabolic rate = plus 97 per cent) was brought to a normal state of metabolism (basal metabolic rate = minus 7 per cent) by Lugol's solution, followed by subtotal thyroidectomy. She later showed a slight secondary rise of metabolism (basal metabolic rate = plus 16 per cent) with omission of Lugol's solution. The marked negative nitrogen balance, present at first, disappeared with treatment. Associated with the negative nitrogen balance there was a large excretion of acid in the urine due to the sulphates and phosphates liberated from the metabolized protein and due to the chloride excretion from the intracellular water of metabolized protein. The fluctuations in the organic acid excretion were insignificant. This marked excretion of acid was met on the basic side by a large titratable acidity value in the urine and by a large total base excretion. These two factors apparently were sufficient so that the ammonia mechanism was not necessary and there was no excess of ammonia excretion. The total base excreted could probably be accounted for by the total base derived from the intracellular water of the metabolized protein plus the calcium excretion from the bones. There was apparently no increase in base derived from extracellular fluids. One is forced to the belief that the marked mobilization of calcium from the bones before treatment helped take care of the large acid excretion, but that the need for base to excrete acid was not the cause of the mobilization, in which case the ammonia mechanism and the excretion of base from extracellular fluid should also have been present. The fact that an increased titratable acidity of the urine was present before operation is probably merely a by result of the increased phosphorus excretion and not evidence of an acidosis.

TAF

William S., aged 45 Admitted January 21, 1927 Discharged March 8, 1927 Diagnosis Myositis ossin

Period number	Calcium metabolism										Phosphorus metabolism (valence assumed at 1.8)										Nitrogen metabolism														
	Weight	Cetologic intake		Fluid intake		Urine		Dried feces		Basal metabolic rate		Urine	Total output		Intake		Balance		Phosphorus equivalent of calcium balance		Urine	Total output		Intake		Balance		Theoretical balance		Urine	Total output		Intake		Balance
kg's	calories	cc	cc	Urine	grams	cc	cc	cc	cc	N/10	cc	N/10	cc	N/10	cc	N/10	cc	N/10	cc	N/10	cc	N/10	cc	N/10	cc	N/10	grams	cc	grams	cc	grams	cc	grams		
I	85.7	7.019	8.523	4.385	96	+15	125	417	169	-248	-129	1,150	1,570	1,360	-210	-283	32.7	35.8	31.2	-4.6															
II	85.6	7.028	8.523	4.220	59		126	338	169	-168	-88	830	988	1,360	373	-51	27.0	30.1	31.2	1.1															
III	84.7	7.035	8.523	4.175	100	-6	143	454	169	-284	-148	880	1,314	1,360	46	-138	27.8	30.9	31.2	3.3															
IV	84.6	7.035	8.523	5.180	60		226	452	173	-278	-145	1,640	1,920	1,360	-560	-419	36.3	39.4	31.2	-8.2															
V	84.1	7.046	8.523	4.915	35		545	667	173	-494	-258	1,750	1,946	1,360	-586	-379	31.7	34.8	31.2	-3.6															
VI	83.7	7.035	8.523	5.070	56		646	906	173	-730	-382	1,430	1,656	1,360	-296	-689	37.3	40.4	31.2	-9.2															
VII	83.1	7.035	8.523	4.835	72		635	920	173	-746	-390	1,470	1,866	1,360	-506	-579	33.8	36.9	31.2	-5.1															
VIII	82.9	7.035	8.523	5.110	29		735	880	169	-710	-370	1,242	1,410	1,360	-50	-484	31.5	34.6	31.2	-3.4															
IX	82.5	7.035	8.523	5.325	74		647	859	173	-686	-360	1,420	1,630	1,360	-270	-408	29.6	32.7	31.2	-1.5															
X	82.2	7.035	8.523	5.525	102		656	913	177	-736	-384	1,540	1,785	1,360	-425	-448	30.0	33.1	31.2	-1.9															
XI	82.4	7.035	8.523	4.640	124		251	492	177	-315	-164	1,200	1,570	1,360	-210	-191	28.9	32.0	31.2	-0.1															
XII	82.0	7.035	8.523	5.625	109		153	363	180	-182	-95	730	1,047	1,360	313	-90	28.0	31.1	31.2	0.1															
XIII	82.0	7.035	8.523	5.090	95		89	336	177	-159	-91	640	1,012	1,360	348	16	24.9	28.0	31.2	3.1															
XIV	82.0	7.035	8.523	4.860	89		52	285	184	-101	-53	635	1,012	1,360	348	151	22.0	25.1	31.2	6.1															

11  
ns.

of nitrogen balance	Total base metabolism										Sulphur	Chloride	Blood serum	Medication	
	Urine	Total output	Intake	Balance	Theoretical balance	Isotopic sulphur urine	Total sulphur intake	Urine	Intake	Ammonia					
$\frac{cc}{10}$	$\frac{cc}{10}$	$\frac{cc}{10}$	$\frac{cc}{10}$	$\frac{cc}{10}$	$\frac{cc}{10}$	$\frac{cc}{10}$	$\frac{cc}{10}$	$\frac{cc}{10}$	$\frac{cc}{10}$	$\frac{cc}{10}$			$\frac{mgm}{100 cc}$	$\frac{mgm}{100 cc}$	
154	-186.4	720.5	961.6	135	174	-434	965.1	671.3	245.3	852	507	862	729	10.9	3.3
37	44.5	000.6	861.6	135	274	-124	830.1	671.3	780.3	852	539	621	740	11.3	3.5
10	12.5	330.6	622.6	135	-487	-272	822.1	671.3	663.3	852	488	659	117	12.2	3.2
274	-531.6	240.7	263.6	135	-1.128	-609	847.1	671.3	780.3	852	610	498	1.081	10.6	3.7
120	-145.6	590.7	014.6	135	-879	-639	958.1	671.3	850.3	852	898	502	1.432	11.3	3.5
307	-371.5	430.6	162.6	135	-27	-1.103	1.072	1.671.2	910.3	852	740	400	1.158	12.2	2.4
190	-230.5	380.6	446.6	135	-511	-976	1.080	1.671.3	655.3	852	767	244	786	11.5	2.4
113	-137.5	940.6	303.6	135	-368	-847	920.1	671.3	910.3	852	813	382	1.065	12.9	2.6
50	-61.0	040.6	894.6	135	-759	-147	765.1	671.3	780.3	852	473	300	848	11.3	3.3
63	-77.6	970.8	010.6	135	-1.875	-813	845.1	671.4	251.3	852	551	155	1.060	12.7	2.8
27	-33.3	450.4	848.6	135	1.287	-348	857.1	671.2	428.3	852	748	339	52	12.0	3.0
4	4.0	000.7	101.6	135	-966	-178	718.1	671.4	118.3	852	819	242	1.495	10.7	4.0
107	129.6	265.7	612.7	335	-275	-30	650.1	671.3	103.3	852	503	-458	1.915	120 cc. M. sol NaHCO <sub>3</sub>	
204	246.6	680.7	986.7	935	-51	145	634.1	671.3	392.3	852	320	-874	1.460	130 cc. M. sol NaHCO <sub>3</sub>	

## PART III

*The effect of parathyroid extract administration on the total acid-base metabolism of an essentially normal individual*

This study has been discussed in a previous publication (2). As the data was never published, however, it is included here in table 11 and chart 3. It furnishes an opportunity to compare the effects of the parathyroid hormone with that of the thyroid hormone.

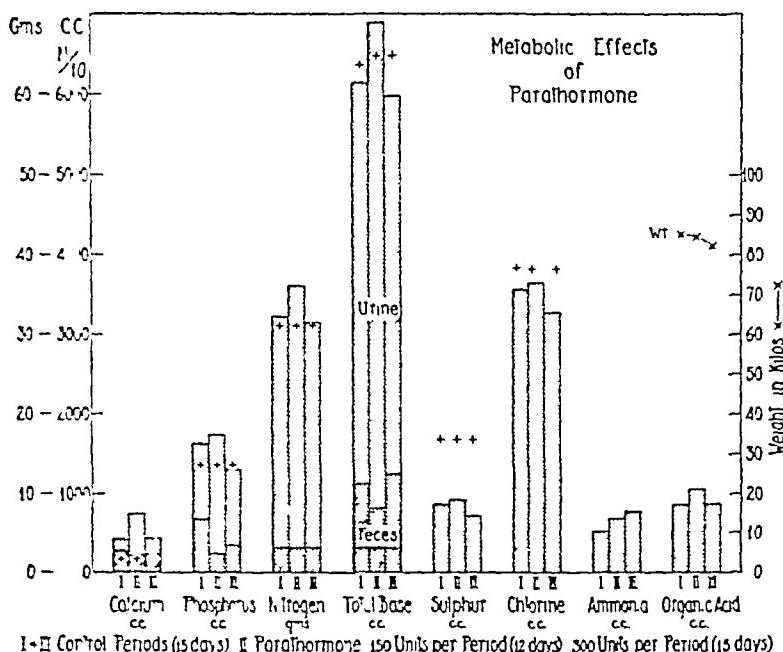


CHART 3

The patient was a laborer of 45 who received parathormone injections in the hope (which was unfulfilled) that an ossified hematoma of the thigh might be decalcified. He had a constant food and fluid intake throughout the duration of the investigation—14 three-day periods. During periods IV–X he received parathormone injections. During the final periods (XIII and XIV) he received sodium bicarbonate.

A detailed discussion of the results will not be repeated here. The outstanding effect of parathormone was on the calcium and phos-

phorus excretions and none of the other fluctuations were sufficient to seem fundamental. There was a tendency for the water, chloride, and total base excretion to increase during the periods of parathyroid administration. This was so slight that it would not deserve comment had there not been a marked reduction of these elements in the first period following cessation of the drug, suggesting that the previous rises were not coincidental. The slight increase in the protein metabolism as shown by the increased nitrogen and sulphur excretions was probably coincidental for it did not occur in the other normal individuals to whom we have given parathormone. Brehme and Gyorgy (19) stress the increased ammonia excretion after parathormone administration but the increase in this observation is certainly of small magnitude. The decrease in titratable acidity in the urine is very surprising, occurring at a time when the phosphates are much increased in the urine. The effect of sodium bicarbonate was what would be expected and needs no analysis here.

In summary it can be said that the parathyroid hormone had very little effect on the electrolytes of the urine except its well known effect on calcium and phosphate, that it did appear to increase slightly the excretion of body fluids with their salts as shown by an increased water, base, and chloride excretion, but that there was no evidence that it upset in any quantitative manner the acid base balance of the body. In spite of an increased phosphate excretion in the urine there was a decreased titratable acidity under parathormone administration.

#### GENERAL SUMMARY AND CONCLUSIONS

The object of this study has been only partially fulfilled. We had hoped to determine the cause of the stimulating action of the thyroid hormone on calcium phosphate excretion but have merely ruled out certain possibilities. That the thyroid hormone does not act by stimulating the parathyroid glands seems evident from the lack of any marked effect of the thyroid hormone on serum calcium and serum phosphorus. That the thyroid hormone brings about mobilization of calcium phosphate to assist in the excretion of acid metabolites, a possibility which seemed a priori very probable, we believe we have likewise disproved. The evidence against this latter supposition is twofold. The marked increased excretion of acid resulting from thy-

roid administration in a case of myxedema was not sufficient to cause the excretion of base dissolved in extracellular body fluids even to a limited extent and the increased acid excretion in the case of hyperthyroidism was not sufficient to cause an increase in ammonia excretion or an increase in excretion of base dissolved in extracellular body fluids. One is forced to the supposition that the thyroid hormone exerts some specific action on the calcium-phosphate metabolism, which is the same as saying we have not found how it acts.

Certain speculations occur to one as possibly pertinent in the explanation of the action of the thyroid hormone on calcium phosphate mobilization from the bones. It seems not impossible that there may be a tissue acidosis resulting from increased cellular activity which is not reflected in the blood stream but which may increase the absorption of calcium from the bones. This is pure speculation. A second explanation is the possibility that the increased blood flow in hyperthyroidism is a factor in the increased calcium excretion. Investigators agree that the total blood flow is increased in hyperthyroidism and decreased in hypothyroidism, roughly proportionately to the metabolism (20, 21, 22). Since calcium is normally present in the blood stream well above its threshold for excretion (23), it would follow that the amount excreted by the kidney (and possibly by the gut) would be proportionate to the blood flow to these parts. Thus with a doubling of the metabolism one might expect an increase of the calcium excretion, provided that the increase in blood flow to the kidney is proportionate to increase in total blood flow. That increase in blood flow is not the entire explanation is shown by the over six-fold increase in calcium excretion in the urine in the case of exophthalmic goiter here reported. Such an explanation, furthermore, would not explain the rises, slight but definite, produced by the thyroid hormone on serum calcium and phosphorus. A third possibility is that the phenomenon may be associated with an increase in permeability of the tissues. Petersen and Levinson (24) in a recent review of this subject found "a striking increase in capillary permeability in exophthalmic goiter" and suggest this as the cause of the increase in calcium excretion. Since calcium diffuses with difficulty, any marked change in permeability of tissues might bring about a marked change in calcium excretion. This explanation, again, fails to account for the

changes in blood values Finally, there may be a combination of several factors at work.

As an interesting by-result of the investigation, it appeared that the protein lost as a result of administration of thyroid to a patient with myxedema was dissimilar from protein lost during starvation or during thyrotoxicosis Whereas hyperthyroidism is associated with the excretion of intracellular fluid with electrolytes in amounts depending on the destroyed protoplasm, these electrolytes seemed to be largely absent in myxedema If as Boothby, Sandiford, Sandiford, and Slosse (16) suggest, administration of thyroid decreases deposit protein, it would appear that deposit protein does not have the same relationship to intracellular water as ordinary structural protein

#### CLINICAL DATA

*Case I* Mrs L C, Massachusetts General Hospital No 281823 Clinical diagnosis Myxedema The patient's hospital entries were from February 18 to April 5, 1927, and from April 18 to April 27, 1927 She was a white, married woman of 63 who entered complaining of swelling of her extremities of 10 months' duration, orthopnea, and dyspnea. She had become mentally and physically sluggish

*Physical examination* Showed the following abnormalities Appearance was typical of myxedema The face and eyes were puffy and the skin coarse and dry The edema did not pit much on pressure. Her sparse hair was fine and dry Her tongue was large and her speech thick and deliberate The heart was enlarged to the left

*Laboratory data (before giving thyroid)* Red blood cells averaged 3,700,000 per cubic millimeter Nonprotein nitrogen on whole blood 60 mgm. per 100 cc, 30 mgm. per 100 cc. Wassermann reaction negative Determinations by Dr Arlie V Bock Oxygen capacity of whole blood 16.0 volumes per cent  $\text{CO}_2$  content of whole blood at 40 mm. pressure 51.5 volumes per cent. Estimated pH of arterial blood 7.50 Blood flow 2.2 liters per minute. Urine negative

*Electrocardiogram* Normal rhythm Small complexes. T inverted (minus 1 mm.) On her second admission to the hospital the electrocardiogram was normal with T<sub>3</sub> plus 2.5 mm

*X ray* Heart increased in size to both right and left. Total width 14.1 cm Internal diameter of chest 25.5 cm

*Diet* The daily diet consisted of Total calories, 1420, calories per kilogram 19, protein per kilogram, 0.81 gram. Diet averaged 100 cc. N/10 of excess acid over base in the ash

*Case II* Miss Emma F, Massachusetts General Hospital No 282948 Clinical diagnosis exophthalmic goiter She remained in the hospital from April

14 to June 5, 1927 The patient was a white, unmarried, American governess of 28 During the previous nine months she had become progressively more nervous and had lost 17 lbs For two months, after an attack of laryngitis, she had noticed a large appetite, marked tremor, perspiration, palpitation, and a progressively enlarging goiter For one month she had noticed easy fatigability and a huskiness of her voice with slight dysphagia For two days, stopping five days before entrance, she had taken 15 drops of a colorless solution of iodine daily

*Physical examination* Showed the following abnormalities A large, hard, smooth symmetrical enlargement of the thyroid over which a systolic thrill and a systolic and diastolic bruit could be noted There was a definite lid lag The heart was large with a rapid rate and a loud systolic murmur, without a thrill, at both apex and base The pulse rate remained above 120 before operation Blood pressure was 140/70

*Laboratory data* Urine Normal except for traces of sugar in three tests Blood morphology normal except for a relative lymphocytosis Nonprotein nitrogen on whole blood 35 mgm per 100 cc Blood sugar 104 mgm per 100 cc Wassermann negative

X-ray examination showed a normal chest except for a heart increased in size both to the right and left Transverse diameter 13.5 cm Chest diameter 24.5 cm

*Operation* (By Dr Edward Richardson) A very large, horseshoe-shaped, symmetrical gland was found About two grams of the right lobe and five grams of the left lobe were left behind A small pyramidal lobe was removed

*Pathological report* (By Dr H F Hartwell) The removed tissue weighed 180 grams No parathyroids were found after careful search Microscopic examination showed proliferation of follicles and extensive hyperplasia of epithelium There was little colloid The stroma contained an increased amount of fibrous tissue and was infiltrated with mononuclear wandering cells

*Course in Hospital* During her stay in the hospital she had a temperature of 102° on the twelfth day after entrance (fourth period), due to a throat infection For seven days after her operation she had a fever up to 102°—higher for the first five days

Her basal metabolism and clinical condition, more than a year and a half after this operation, were normal She appeared completely recovered

*Diet* Her diet consisted daily of the following Total calories, 3000, calories per kilogram 45, protein per kilogram, 1.5 gram Diet averaged 81 cc N/10 of excess acid over base in the ash Water intake was 3000 cc per day

*Case III* Mr William S, Massachusetts General Hospital No 281245 Clinical diagnosis Myositis ossificans The patient stayed in the hospital from January 21 to March 8, 1927 He was a white, married laborer of 45 years Three months before entrance he had been hit in the left thigh The leg had become swollen, but he had been able to walk comfortably X-ray examination showed a marked deposit of calcium in the muscle

*Physical examination* Showed the following abnormalities On the lower third of the left upper leg there was a hard, irregular lump apparently attached to the soft parts

*Laboratory data* Blood morphology normal Wassermann reaction negative. Basal metabolism January 25, plus 15 per cent, February 1 minus 6 per cent February 28, minus 7 per cent Urine negative

*Progress* The myositis ossificans was unaffected by the treatment.

*Diet* His diet consisted daily of the following Total calories, 2275, calories per kilogram 27, protein per kilogram, 0.765 gram Diet averaged 84 cc. v/10 excess base in the ash Water intake was 2600 cc. per day

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## STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM

### XII THE EFFECT OF THE INGESTION OF ACID-PRODUCING SUBSTANCES

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### ERRATA

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*Article by Harrop and Barron*

*Page 578, line 5 from bottom "(Harrop and Barron (7))" should read "(Barron (7))"*

*Page 580, line 11 "(Harrop and Barron (7))" should read "(Barron (13))"*

*Page 587, reference 7 shou'd read "Barron, E S G , Bull Johns Hopkins Hosp To be published The Nature of the Van den Bergh Reaction " Reference 13 should be added "Barron E S G , Medicine, 1931, x, 77 Bilirubinemia "*

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THE EXPERIMENTS OF GIVENS AND BREWER (3) and of GIVENS (4) upon the effect of base and acid on the general metabolism tended to minimize variations in calcium excretion resulting from this factor. Subsequently, Goto (5), using rabbits, demonstrated depletion of the skeleton by repeated doses of hydrochloric acid. Lamb and Evvard (6) demonstrated increased urinary calcium

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excretion in pigs following the administration of mineral acid, but not after feeding oxidizable organic acids Shohl and Sato (7) showed that the feeding of hydrochloric acid decreased the storage of calcium by growing children, and demonstrated the reverse effect of sodium bicarbonate These effects were also noted by Zucker (8)

The fate of inorganic acid in animals has been followed by Fiske and Sokhey (9) and by Fiske and other collaborators (10) Stehle and McCarty (11) extended the former's work on ingested hydrochloric acid to man Bogert and Kirkpatrick (12) specifically studied the effect of acid-forming and base-forming diets upon calcium metabolism in man, but their interesting experiments were confused by lack of constant calcium intake throughout All of these investigators found that an increase in calcium excretion followed administration of excess acid

Less importance has been attached to endogenous acid metabolites Nevertheless, Nelson (13) has pointed out the effect of ketogenic diets on calcium metabolism Her results confirm the earlier work of Sawyer, Baumann, and Stevens (14) on the effects of ketosis These studies, together with an investigation on the inorganic salt metabolism in diabetic acidosis (15), have furnished ample evidence that acid metabolites, which arise from body tissues, are likewise important in regulating or influencing calcium excretion The classical work of Benedict (16) on subject L, during the latter's thirty-one-day fast, has already been mentioned from this point of view (17)

The influence of various hormones upon calcium metabolism has already engaged the attention of those interested in this field, but the mechanism by which the minute amounts of these substances produce their specific effects upon bone metabolism remains to be solved The same is true of (exogenous) accessory food substances It is very important, therefore, in view of the gross changes in body tissues which these specific chemical regulators produce, to understand the effect which endogenous acid catabolites may exhibit The excessive breakdown of protein in hyperthyroidism is a case in point

In order to establish the quantitative significance of this factor, it was first necessary to determine the basal level of endogenous calcium metabolism in each (human) subject studied while taking a low calcium diet Thereafter, acid- or alkali-producing substances could be added

and their effects noted as superimposed upon the basal stream of calcium excretion.

In this work little stress will be laid upon the calcium and phosphorus content of the blood serum because these values showed little effect resulting from change in diet. The height of these levels, in normal subjects at least, had essentially no influence upon the calcium stream. Much more emphasis has been placed upon the excretion of acid in the urine because a consideration of this value (or of its components) offers one of the best guides to a quantitative interpretation of calcium balances. This is the more important because, as will be demonstrated, the excretion of fecal calcium has remained essentially constant despite the various dietary changes upon which these experiments are based, wherefore it may be concluded that variations of bowel activity including increased excretion of calcium or deficient assimilation (such as have been reported in studies on rickets or in ergosterol feeding) may be neglected in this work.

#### EXPERIMENTAL PROCEDURE AND METHODS

The general procedure was essentially that described in a former paper (18). Most of the patients were given a constant diet low in calcium (about 100 mgm daily) and moderately low in phosphorus, but adequate with respect to other inorganic salts, fat, carbohydrates, protein, total calories, and vitamins (18). In one patient, the effects of acid administration were observed first on this low calcium diet and then on a diet containing about 700 mgm Ca per day, which was sufficient to give a definite positive calcium balance. In all cases on a given diet the same articles of food were taken each day so that whatever errors in calculation might exist would remain as nearly constant as possible throughout. The basal diet was so constituted as to give a neutral inorganic residuum when oxidized,—such a diet being described as a potentially neutral diet. Except in experiments in which the effect of an acid diet was studied, the same potentially neutral diet was used throughout the whole period of observation of each patient.

Although by calculation these diets were potentially neutral, nevertheless, in all cases the patients excreted an appreciable amount of "total acid" as judged by ammonia and titratable acidity of the

urine. The "total acid"<sup>2</sup> output was highest in a patient whose stools were very large, due to deranged fat absorption and it was found that the titratable alkalinity of the fecal ash (which probably neutralized fatty acids in large measure) was about equal to the ammonia plus titratable acidity of the urine. For different persons on the same type of neutral diet there was considerable variation in the "total acid" output of the urine. It is probable that for a given diet this "total acid" output varies with the amount of excess alkali in the fecal ash. From the point of view of acid-base metabolism, the potential acid effect of the diet in a given case can be gauged qualitatively by relative changes in the "total acid" output of the urine. Accordingly, the ammonia and titratable acidity of the urine were measured daily in all subjects studied.

Specimens for general purposes were collected and mixed in three-day periods as described elsewhere (18). Care was taken to obtain stools regularly and constipation was prevented by frequent small doses of cascara. In many cases the stools were approximately equal in weight and inorganic constituents. Occasionally, however, a very large stool in one period followed an unusually small one for the preceding period and it was obvious that there had been a delay in evacuation. In one instance (BE-Case IV) a definite constipation with small hard stools persisted for about three periods in which fecal inorganic content, including calcium, was very low. In the succeeding periods there was no unusually large fecal excretion of calcium or other inorganic salts, thus indicating that there had been an increased absorption from the large bowel. It was also noticed in some other cases, not reported in this paper, that the calcium content of the feces tended to be higher on changing to a diet with great fecal residue and lower when residue was small. Except in the instance already noted, these factors were well controlled during this investigation.

#### METHODS

All determinations were done in duplicate and repeated if satisfactory checks were not obtained. The methods used for titratable

<sup>2</sup>Throughout this paper the term "total acid" output refers to the sum of ammonia plus titratable acidity in the urine. The term "titratable acidity" means the titratable acidity minus CO<sub>2</sub> as described in Paper VIII of this series, J. Clin. Invest., 1931, v. 187.

acidity, ammonia, phosphorus, chloride, and total base were those of Henderson and Palmer, Folin, Fiske and Subbarow, Van Slyke, and Fiske respectively, with certain minor modifications as formerly described (18) Calcium determinations in blood, urine, and feces were made by Fiske's method (19) This method has proven most satisfactory under all conditions The duplicates seldom varied as much as 2 per cent and the calcium could be reprecipitated and redetermined with nearly identical results Inorganic sulphate only was determined (Tables 1, 2 and 3) by Fiske's benzidine method

#### EXPERIMENTS AND RESULTS

The first experiments were planned to show the relative effects of ingestion of (1) a potentially acid diet, (2) varying quantities of alkali and (3) varying amounts of  $\text{NH}_4\text{Cl}$  on the calcium and phosphorus metabolism In some instances dietary changes were made after too short an interval as we had not at that time realized the necessity of longer control periods and of the length of time required before a steady state could be established The general plan in the first three cases was very similar and they are, therefore, presented together

*Case I* (AN, aged 16, weighing 34 kilos) suffered from a marked structural scoliosis After a 15 day control period on a neutral, low calcium diet and prior to operative interference he was placed at complete rest in a plaster shell for several weeks Two periods were allowed for control in this state of inactivity, following which the diet was changed to one of about 700 cc. N/10 potential acidity per day for three periods, then while still at rest and on the former neutral control diet, the effect of ingestion of  $\text{NH}_4\text{Cl}$  and  $\text{NaHCO}_3$ , was observed Six months after a spinal fusion for correction of his deformity he was readmitted and the effect of ingested  $\text{NH}_4\text{Cl}$  once more observed.

*Case II* (DA), aged 37, weighing 58 kilos, and *Case III* (ST), aged 46, weighing 51 kilos, were both recovering from lead poisoning A similar series of experiments were made as on AN, the details and results of which are presented in tables 2 and 3

Later the effect of graded doses of  $\text{NH}_4\text{Cl}$  was observed for longer intervals, first on a low and then on a higher calcium diet on a patient, (BE) aged 58, weighing 55 kilos, who suffered from chronic sciatic neuritis Results are presented in table 4

All these patients cooperated willingly, took the diet cheerfully and completely and seemed happy throughout the observation

TABLE I  
AN—Case I

Diet	Period	Total acid excretion		Nitrogen		Calcium		Phosphorus		Total base		Chloride		Sulphite				
		cc N/10		cc N/10		Urea		Urea		Urea		Urea		Urea				
		Urea	Urea	Urea	Urea	Urea	Urea	Urea	Urea	Urea	Urea	Urea	Urea	Urea	Urea			
Control	I and II discarded	154	482	636	17	2.23	5.0	18.0	15—0	0.021	1.60	4.0	0	23.3	790	611		
		10	—255	942	687	17	0.23	5.0	19.0	18—0	0.061	1.50	4.0	0	24.3	658	751	
Control	neutral low calcium	10	—728	1,461	733	17	0.23	5.0	16.0	16	0	0.01	0.60	4.4	0	29.3	553	674
		10	—617	1,132	515	18	3.23	5.0	27.0	15—0	1.10	7.80	4.0	0	61.3	997	619	
Control	Complete rest	10	—635	1,201	566	16	7.23	5.0	25.0	18—0	1.10	8.40	4.3	0	52.3	370	837	
		10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Acid, low in calcium	VIII	2,156	177	1,258	1,435	30	0.48	5.0	63.0	17—0	50.1	71.0	5.0	0	67.3	653	583	
	IX	2,069	—808	2,136	1,328	34	0.46	6.0	71.0	14—0	57.1	65.0	3.9	0	50.4	74.0	44.6	
Control + NH <sub>4</sub> Cl	X	2,069	—992	2,549	1,557	35	6.46	6.0	71.0	16—0	57.1	83.0	4.0	0	31.5	285	693	
	XI	4,112	—633	3,834	3,201	32	1.29	9.1	13.0	19—1	0.11	9.20	3.5	—0	47.5	839	712	
Control	XII	3,376	—434	3,982	3,548	25	9.28	2.1	16.0	21—1	0.51	8.70	4.4	—0	52.1	390	910	
	XIII	15	—1,008	2,597	1,589	19	4.23	5.0	6.60	21—0	5.61	3.50	4.9	—0	06.2	948	934	
Control + NaHCO <sub>3</sub> + sodium citrate	XIV	10	—1,493	1,687	194	18	9.23	5.0	3.80	17—0	23.1	2.20	4.5	0	12.4	223	511	
	XV	3	—680	1,437	757	15	3.23	2.0	2.40	20—0	12.1	1.19	4.4	0	10.3	912	959	
Control + NaHCO <sub>3</sub> + sodium citrate	XVI	—1,715	—3,216	170	—3,046	16	8.23	5.0	2.50	16—0	10.1	11.0	4.2	0	26.7	418	810	
	XVII	—1,190	—3,996	142	—3,854	15	9.23	5.0	2.20	18—0	0.081	1.20	4.2	0	25.8	970	811	

		Second admission after operation									
		VIII	-7	95	638	733	18	323	20	040	15
		XIX	10	-62	526	464	18	523	50	050	15
Control + 6 grams NH <sub>4</sub> Cl	XX	3 334	702	1,953	2 655	28	327	50	20	14	-0 051
	XXI	3 376	654	2,637	3,291	26	728	20	250	12	-0 071
	XXII	877	536	2,112	2,648	26	028	20	270	16	-0 141
	XXIII	-511	-683	492	-191	22	128	20	040	12	0 131
	XXIV	-135	-297	558	261	24	328	40	020	08	0 181
	XXV	698	286	613	899	25	928	20	020	11	0 161
Control	XXVI	239	196	724	920	22	925	10	030	10	0 161
	XXVII	10	255	660	915	24	323	50	010	09	0 181

Potentially neutral diet Calcium 0.097 gram, phosphorus 0.595 gram, protein 49.4 grams, total calories 1,757 daily  
 Acid diet Calcium 0.097 gram phosphorus 0.964 gram, protein 102.3 grams, calories 2,340, daily

TABLE 1A  
*Serum values*

Period	Plasma CO <sub>2</sub> content milliliters per cent	Serum calcium		Serum phosphorus mgs. per 100 cc.	Serum protein per cent
		mgs. per 100 cc.	mgms. per 100 cc.		
X	68.5	10.3	5.2	7.0	
XII	53.4	9.2	5.2	6.0	
XVII	65.3	10.2	5.1	6.7	
XIX		9.8	4.8	6.9	
XXII	51.2	9.0	4.3	6.7	
XXVII	59.8	8.7	4.6		

TABLE 2  
D 1—Case II

Diet	Period	Total acid excretion												Chloride						Sulphate				
		Nitrogen				Calcium				Phosphorus				Total base			Urine			Bicarbonate		Urine		
Triflatabile acidity + ammonia in urine		Ammonia in urine		Triflatabile acidity + ammonia in urine		Ammonia in urine		Triflatabile acidity + ammonia in urine		Ammonia in urine		Triflatabile acidity + ammonia in urine		Ammonia in urine		Triflatabile acidity + ammonia in urine		Ammonia in urine		Triflatabile acidity + ammonia in urine		Ammonia in urine		
$\text{cc } N/10$		$\text{cc } N/10$		$\text{cc } N/10$		$\text{cc } N/10$		$\text{cc } N/10$		$\text{cc } N/10$		$\text{cc } N/10$		$\text{cc } N/10$		$\text{cc } N/10$		$\text{cc } N/10$		$\text{cc } N/10$				
Control	I	529	862	1,391	29	229	30	180	24	-0	091	430	32	0	444	317	545	1,918	2,920	3,591	1,000	1,263		
	II	544	744	1,288	31	029	30	140	74	-0	551	491	36	-0	665	1,936	-275	3,470	3,591	970	1,263			
	III	434	563	997	22	629	30	090	53	-0	291	010	89	0	293	517	1,253	2,152	2,520	3,591	680	1,263		
	IV	1,418	407	625	1,032	24	742	40	160	58	-0	431	020	90	0	743	990	1,314	1,647	2,850	4,297	750	1,813	
	V	2,111	585	1,389	1,974	31	548	00	130	51	-0	351	530	91	0	396	642	1,104	-929	4,620	4,540	1,190	2,033	
	VI	2,170	508	1,359	1,867	24	349	00	180	51	-0	401	440	90	0	554	983	1,705	278	4,045	4,650	1,040	2,038	
	VII	-2,138	-55	918	863	27	529	30	090	50	-0	241	040	73	0	425	742	1,200	2,010	3,300	3,591	810	1,263	
	VIII	-2,103	-1,199	242	-957	23	827	50	090	40	-0	160	830	87	0	405	725	1,526	1,207	3,250	3,176	680	1,211	
	IX	-7,622	-5,891	292	-5,599	21	529	30	140	51	-0	291	071	15	-0	089	282	1,878	3,022			550	1,261	
	X	-7,304	-6,641	194	-6,147	20	229	30	150	52	-0	330	811	24	0	109	963	1,762	2,168	2,280	3,591	620	1,263	
Control	+ NaHCO <sub>3</sub>																							
Control	+ NaHCO <sub>3</sub> + sodium citrate																							
Control	+ NH <sub>4</sub> Cl																							

Control + NH <sub>4</sub> Cl	XIV	6,736	1,013	3,948	4	961	36	38	70	50	61	-0	752	191	17	-1	218	320	1,445	-3,180	10,700	10,323	
	XV	6,736	859	4,787	5	656	36	7	38	70	730	53	-0	902	030	80	-0	685	7061	1,175	-296	9,580	10,323
Control	XVI	4	260	3,400	3	660	28	0	29	30	160	84	-0	650	841	19	0	121	2141	787	3,584	2,750	3,591
	XVII	4	6092	543	2	603	24	0	29	30	150	45	-0	250	480	97	0	711	3111	1,369	3,905	2,260	3,591
Control + NaH <sub>2</sub> PO <sub>4</sub>	XVIII	4	3101	562	1	872	15	7	29	30	230	47	-0	350	461	06	0	632	8631	1,446	2,276	4,980	3,591
	XIX	2,142*	1	2141	136	2	350	21	529	30	070	34	-0	084	573	01	2	563	361	1,656	4,148	2,590	3,591
Control	XX	2,142*	1	179	699	1	878	21	529	30	060	56	-0	285	913	82	0	415	2932	174	1,698	2,770	3,591
	XXI	242	295	451	746	21	629	30	100	51	-0	272	311	58	-0	846	2951	620	-1,043	4,220	3,591	710	1,263
Control	XXII	4	274	444	718	19	329	30	070	49	-0	210	861	05	0	244	2701	717	598	3,300	3,591	570	1,263
	XXIII	4	245	511	756	20	729	30	070	46	-0	180	691	01	0	464	6401	151	794	3,920	3,591	650	1,263

\* Calculated with reference to pH of the blood

Potentially neutral diet Calcium 0.110 gram, phosphorus 0.730 gram protein 60.7 grams, total calories 2010 daily  
 Acid diet Calcium 0.094 gram, phosphorus 0.964 gram, protein 102.3 grams, total calories 2,340, daily

TABLE 2A  
 Serum values

Period	Plasma CO <sub>2</sub> capacity milliliters per cent	Serum calcium mgs. per 100 cc.	Serum phosphorus mgs. per 100 cc.	Serum protein per cent		Nonprotein nitrogen mgs. per 100 cc.									
				III	VI	VII	VIII	X	XV	XVIII	XX	XXIII			
		9.1		3.6	3.6	5.5	42								
		9.1		3.7	3.7	5.6									
		9.1		4.7	4.7	5.9									
		9.8		3.2	3.2	6.1	25								
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									
		9.8		3.2	3.2	6.1									

TABLE 3  
*Stryck—Case III*

Diet	Period	Total acid excretion		Vitrogen		Calcium		Phosphorus		Total base		Chloride		Sulphate		
		cc N/10	cc N/10	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	
Control neutral diet	I	568	621	1,192	21.9	29.3	0.13	0.31	-0.12	1.08	0.59	0.52	4.351	3,590	3,084	
	II	659	508	1,167	25.4	29.3	0.11	0.35	-0.12	1.52	0.49	0.18	4.666	882.3	410.3, 084	
	III	-33	191	371	862	18.0	27.1	0.06	0.33	-0.08	1.12	0.48	0.50	2.731.1, 036	2,091.1, 823.2, 759	198.1, 314
Acid, low in calcium	IV	2,111	811	570	1,381	30.2	48.0	0.20	0.31	-0.22	1.56	0.54	0.73	3,890	2,635.4, 036	999.2, 322
	V	2,171	787	1,227	2,014	31.2	18.1	0.37	0.37	-0.41	1.62	0.57	0.70	5,600.1, 069	-210.4, 570.1, 113	971.2, 370
	VI	2,171	882	1,431	2,316	33.7	18.1	0.54	0.30	-0.51	1.90	0.53	0.46	5,210	914	275.1, 810.4, 1,113.1, 116.2, 370
Control + NaHCO <sub>3</sub>	VII	-2,138	-57	516	159	26.8	29.3	0.32	0.26	-0.23	1.35	0.37	0.47	6,929	709	918.4, 340.3, 084
	VIII	-2,138	-1,108	101	-1,007	21.5	29.3	0.19	0.33	-0.16	1.32	0.32	0.55	6,569	810	1,177.2, 950.3, 084
Control + NaHCO <sub>3</sub> and sodium citrate	IX	-7.304	-5,991	111	-5,880	22.3	29.3	0.20	0.37	-0.22	1.75	0.66	-0.23	10,651.1, 438	1,630.2, 035.3, 081	523.1, 422
	X	-7.304	-6,107	73	-6,334	20.2	29.3	0.35	0.28	-0.29	1.08	0.51	0.60	12,216	713	763.1, 255.3, 084
Control + 12 grms NH <sub>4</sub> Cl daily	XI	6,736	37.1, 810	1,847	31.1	38.7	0.65	0.12	-0.71	2.12	0.62	-0.55	9.134	795	-3,515.9, 190.9, 816	
	XII	6,736	1,120.5, 737	5,857	38.4	38.7	1.15	0.11	-1.20	2.75	0.53	-1.00	6,435	815	-836.8, 670.9, 816	
	XIII	6,736	1,023.6, 131	7,153	36.1	38.7	1.13			1.76			5,329		9,860.9, 816	
															712.1, 422	

Potentially neutral diet Calcium 0.110 gram, phosphorus 0.729 gram, protein 60.7 grams, total calories 2,010, daily  
Acid diet Calcium 0.097 gram, phosphorus 0.061 gram, protein 102.3 grams, total calories 2,310 grams

TABLE 1A  
Serum values

Period	Plasma CO <sub>2</sub> capacity	Serum values		Nonprotein nitrogen
		mmoles per cent	mgm. per 100 cc.	
IV	68.8		4.4	6.5
VII	62.6	9.5	4.1	6.2
IX	70.1	9.9	5.5	6.5
XI	75.8	10.0	4.9	8.0
XIII	42.3	9.1	3.2	6.1
				39

III - Case IV

12000 121

Diet		Period	Nitrogen				Calcium				Phosphorus				Total Nitrate				
Total	Control		Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	
Control	control	I	17	270	142	712	24	3	29	3	0.08	0.11	0.18	1.22	0.81	0.09	1,260	1,515	602
Control	neutral	II	17	318	115	763	25	4	29	3	0.10	0.17	0.20	1.17	0.93	0.02	1,690	1,571	1,214
Control	low calcium	III	11	177	162	539	24	5	29	3	0.13	0.16	0.05	1.18	0.36	0.57	1,960	571	1,214
Control	high calcium	IV	11	257	168	725	23	7	29	3	0.15	0.19	0.12	1.19	0.97	0.87	1,360	2,085	3,150
Control	low calcium	V	17	387	583	970	23	0	29	3	0.18	0.19	0.12	1.17	0.97	0.87	1,690	2,085	3,150
Control	control	VI	2,220	684	1,103	1,787	29	7	32	4	0.39	0.25	0.31	1.67	0.36	0.07	7,320	515	-1,028
Control	low calcium + 1 gram NH <sub>4</sub> Cl daily	VII	2,218	511	1,266	1,777	21	2	31	7	0.38	0.39	0.32	1.11	0.74	0.41	1,870	1,480	292
Control	control	VIII	2,254	737	1,757	2,194	28	6	30	5	0.18	0.39	0.51	1.78	0.96	-0.045	1,780	1,690	-1,218
Control	low calcium	IX	2,237	728	1,986	2,711	27	7	30	2	0.46	0.22	0.33	1.84	0.64	0.21	3,330	1,050	1,845
Control	high calcium	X	2,251	712	2,051	2,763	26	6	30	4	0.12	0.32	0.10	1.72	0.87	0.11	3,320	1,400	1,532
Control	control	XI	2,251	758	2,187	2,945	27	5	30	1	0.45	0.29	0.40	1.82	0.72	0.16	3,860	880	1,512
Control	low calcium + 6 grams NH <sub>4</sub> Cl daily	XII	3,376	770	2,378	3,118	29	1	32	0	0.54	0.32	0.51	1.91	0.85	-0.06	1,050	1,400	802
Control	control	XIII	3,376	756	2,804	3,560	31	6	32	0	0.65	0.33	0.63	2.12	0.81	-0.23	1,630	1,400	222
Control	high calcium	XIV	3,376	890	2,960	3,850	31	2	32	0	0.62	0.62	2.17	2.17	3,870				
Control	control	XV	3,376	687	2,547	3,231	26	1	36	1	0.93	0.96	0.23	1.85	0.81	0.41	3,230	1,435	1,626
Control	low calcium + 6 grams NH <sub>4</sub> Cl daily	XVI	3,376	614	2,878	3,522	26	6	36	1	1.02	1.08	0.01	1.98	0.66	0.153	1,420	1,530	1,311
Control	control	XVII	3,376	658	2,966	3,621	27	1	36	1	1.03	1.01	0.06	1.98	0.64	0.173	3,910	1,350	1,018
Control	high calcium	XVIII	-35	164	2,297	2,761	23	7	31	7	0.81	1.03	0.22	1.51	0.71	0.87	2,290	1,520	2,112
Control	neutral	XIX	-35	319	1,164	1,683	19	1	31	7	0.53	0.79	0.79	1.24	0.50	1.352	1,170	1,030	3,052
Control	high calcium	XX	-35	68	518	586	16	9	31	7	0.38	0.97	0.76	1.22	0.56	1.313	3,590	1,355	1,307
Control	control	XXI	-35	28	357	385	18	2	31	7	0.25	1.19	0.68	1.47	0.67	0.95	1,535		

Control neutral low calcium diet Calcium 0.108 gram, phosphorus 0.706 gram, protein 60.7 grams, calories 1,995, daily  
Control neutral high calcium diet Calcium 0.701 gram, phosphorus 1.031 grams, protein 61.6 grams, calories 2,150 daily

TABLE 4A  
Serum values

Period	Plasma CO <sub>2</sub> content millies per cent	Serum calcium		Serum phosphorus		Serum protein per cent
		mmoles per 100 cc.	mgm. per 100 cc.	mmoles per 100 cc.	mgm. per 100 cc.	
VII	52.9	9.3	3.8			
IX	54.7	10.0	3.7			
XI	48.5	9.2	3.1			
XV	53.1	9.0	3.7			
XVII	63.0	9.8	3.0			
XIX		9.0	3.0			
XXI	55.1	9.2	3.6			
			3.6			

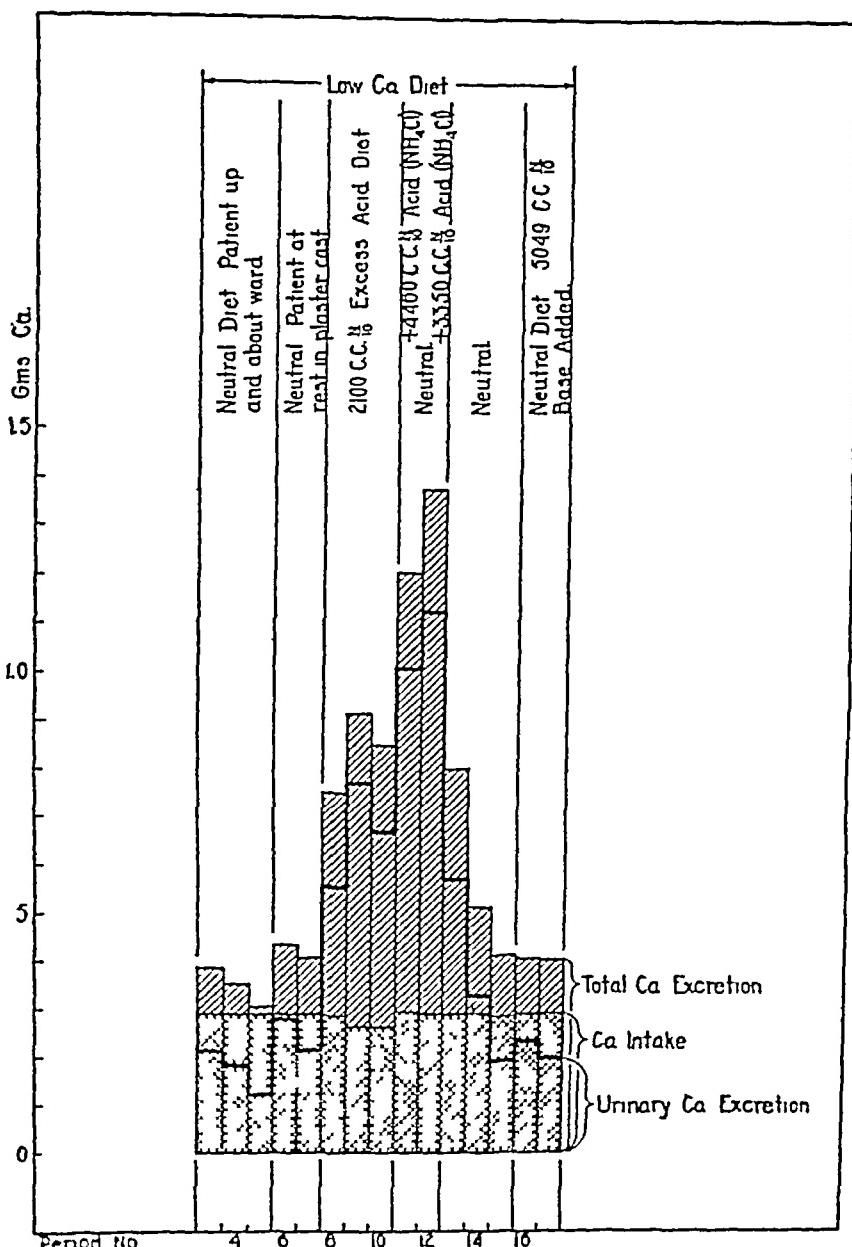


FIG 1 CALCIUM EXCRETION PER THREE-DAY PERIOD

Calcium excretions per three-day period are divided into fecal and urinary components. The calcium intake, as shown, remains nearly constant. The potential acidity of the ingesta (including both food and medication) is indicated at the top of the figure.

## COMMENT

On ingestion of a potentially acid diet or of  $\text{NH}_4\text{Cl}$  there was in all cases a prompt though gradual increase in the excretion of calcium in the urine, which reached a maximum usually in the second or third period and then remained at this high level for the whole duration of acid administration. In the after-periods the urine calcium fell gradually, requiring from 6 to 9 days to return to the basal level. It was notable that under the conditions of these experiments the fecal calcium remained remarkably constant, showing no appreciable change even when the calcium content of the urine was increased tenfold.

The effect of ingestion of an acid diet was essentially similar to that of ingested inorganic acid, subject to certain modifications. Since the protein of the food contributes much to the excess acid of this diet, the "acid effect" depends in large measure on the nitrogen balance. In the case of DA (Case II, periods IV, V, and VI) for instance, on the acid diet there was a gross positive nitrogen balance and the acid effect as judged by the "total acid" excretion of the urine was correspondingly lessened. His urinary calcium excretion, accordingly, did not increase on ingestion of this diet to the same extent as did that of AN and ST, who utilized most of the protein fed as fuel. It may be noted, however, that in none of the three was the acid effect of the diet, as judged by the ammonia plus titratable acidity of the urine, quite as great as calculated. The response of the urinary calcium to the acid diet and administration of  $\text{NH}_4\text{Cl}$  in AN, (Case I) is graphically represented in figure 1. This shows clearly the great increase that occurred in response to both types of acid administration.

That the effect of the acid diet was not due to any specific influence of the greater protein content apart from its potential acid value has been demonstrated by other experiments (20) in which it was shown that when sufficient  $\text{NaHCO}_3$  was administered to neutralize the acid effects of a high protein diet, no increased excretion of calcium occurred. Only about one fourth of the calculated excess acid in these diets was due to an increased phosphorus content.

*The quantitative response to acid ingestion*

There are some interesting observations with regard to the actual quantitative increase in calcium excretion in response to the ingestion

TABLE 5  
*Response of urinary calcium excretion to ingestion of NH<sub>4</sub>Cl\**

Case	Basal calcium excretion in urine	Calcium in urine					
		4 grams NH <sub>4</sub> Cl		6 grams NH <sub>4</sub> Cl		12 grams NH <sub>4</sub> Cl	
		Calcium in urine	Increase over basal calcium excretion	Calcium in urine	Increase over basal calcium excretion	Calcium in urine	Increase over basal calcium excretion
	grams	grams	per cent	grams	per cent	grams	per cent
An—Case I							
1st admission, weight 34 kilos	0.258			1.160	450		
2nd admission (retaining calcium), weight 36 kilos	0.052			0.246	473		
BE—Case IV							
Weight 55 kilos, low calcium diet	0.152	0.449	295	0.635	418		
Higher calcium diet	0.246			1.027	417		
DA—Case II, weight 58 kilos	0.095	0.205	216			0.726	764
ST—Case III, weight 51 kilos	0.085					1.126	1,325
Ammonia in urine							
	Control	4 grams NH <sub>4</sub> Cl	6 grams NH <sub>4</sub> Cl	12 grams NH <sub>4</sub> Cl			
AN—Case I	cc N/10	cc N/10	cc N/10	cc N/10			
1st admission	1,166		3,982†				
2nd admission	526		2,637†				
BF—Case IV							
Low calcium diet	471	1,995	2,882				
High calcium diet	357		2,922				
DA—Case II	550	1,344				4,787	
ST—Case III	439					6,141	

\* Transitional period omitted in making calculations

† On this first admission, there was an actual overproduction of ammonia so that the urine was alkaline throughout. The corresponding values for "total acid" excretion are 3,548 and 3,290 respectively.

of acid. For instance, on the first admission of AN (Case I, scoliosis) the urinary calcium was moderately high, there was a negative calcium balance and on ingestion of 6 grams of  $\text{NH}_4\text{Cl}$  per day the urinary calcium increased fourfold till it was over 1 gram per period. On his second admission, 6 months after an orthopedic operation, the urinary calcium was low and even on the low calcium intake there was a definite positive calcium balance. The calcium metabolism in short was essentially like that of a growing child. On daily ingestion again of 6 grams of  $\text{NH}_4\text{Cl}$  the "total acid" of the urine increased to about the same level as on the former admission. The calcium output in the urine, however, although it increased fourfold did not exceed 0.25 gram and was in fact about the same as that of the basal periods of his first admission. It seems probable that, under the altered metabolic conditions following the spinal fusion but on the same calcium intake, his body was retaining more calcium and allowing only a small amount to be discharged in the outflowing stream. Yet, it is interesting that the same amount of  $\text{NH}_4\text{Cl}$  in the two instances gave rise to about the same proportionate increase of urinary calcium.

In looking over the rest of the data of different "normal" subjects from this point of view, it appears that although there was a great difference in the basal level of urinary calcium excretion and also great variation in the amount of extra calcium excreted in response to a given dose of  $\text{NH}_4\text{Cl}$ , yet in each case the increase in the output of calcium was roughly proportional to the initial basal level and to the amount of  $\text{NH}_4\text{Cl}$  ingested. This is well shown by data collected in table 5.

Thus, in BE (Case IV) on ingestion of 6 grams of  $\text{NH}_4\text{Cl}$  daily the urinary calcium was 0.635 gram on a low calcium diet, (periods XIII and XIV), and 1.03 gram on a high calcium diet (periods XVI and XVII). This represents an increase of about 400 per cent above the control level for the corresponding diet in each instance. This proportionate increase is of essentially similar magnitude to that which occurred in AN on each admission in response to the same amount of  $\text{NH}_4\text{Cl}$ . The response to 4 grams  $\text{NH}_4\text{Cl}$  in DA (Case II, periods XII and XIII) was a 216 per cent increase in urinary calcium, and in BE (Case IV, periods VIII-XI) a 295 per cent increase although in

BE the actual increase in grams of calcium in the urine was more than double that which occurred in DA

In figure 2, the relation of the percentage increase of calcium excreted in the urine to the amount of  $\text{NH}_4\text{Cl}$  ingested per kilo is graphically represented. This series of points approaches the form of a smooth J-curve.

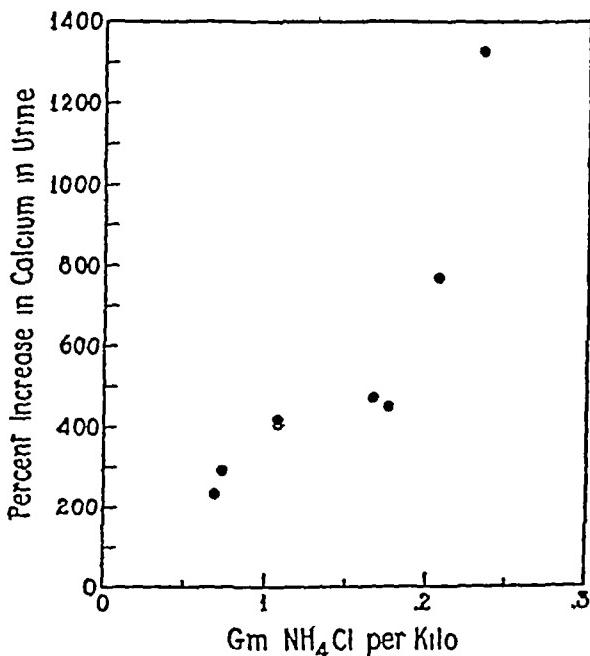


FIG 2 THE EFFECT OF AMMONIUM CHLORIDE INGESTION UPON URINARY CALCIUM EXCRETION

The increase in urinary calcium excretion over the basal level is expressed as a percentage. The corresponding amounts of ammonium chloride fed are given in milligrams per kilo of body weight.

It may be that the factors tending to increase calcium excretion as a result of ingestion of  $\text{NH}_4\text{Cl}$  are limited by the factors already regulating the urinary output of calcium. The result, therefore, might be viewed as a proportionate increase of the outflowing stream, rather than as a quantitative response in calcium excretion to a definite amount of acid ingested.

*The rôle of the increased calcium excretion in neutralizing excess acid ingested*

Although the ingestion of excess acid has a pronounced effect on the urinary calcium excretion and on the calcium balance, nevertheless,

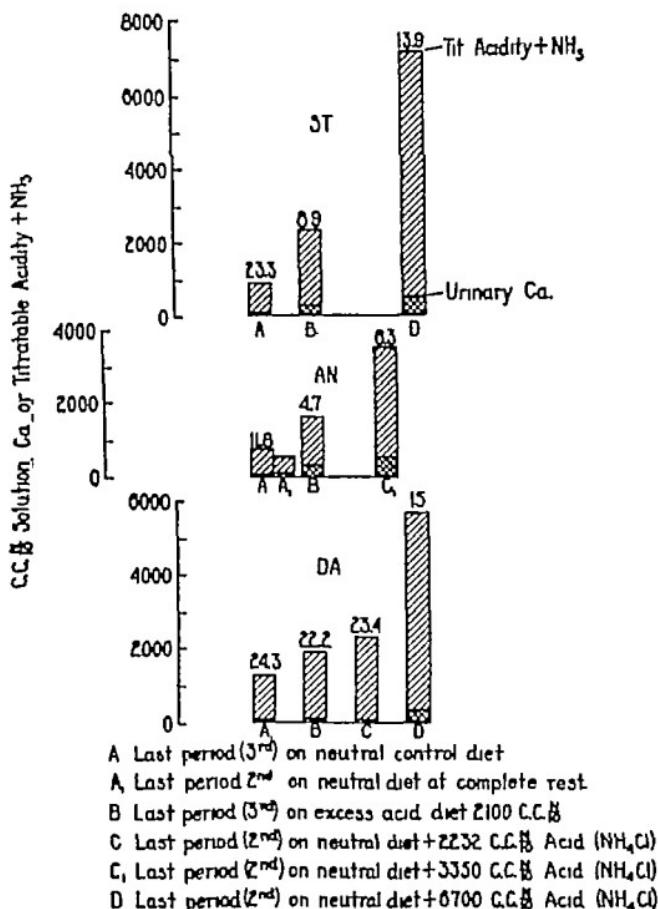


FIG. 3 THE EFFECT OF ACID DIETS AND ACID SALTS UPON THE ACID AND CALCIUM EXCRETION IN THE URINE

the increased calcium of the urine plays but a small part in neutralizing the excess acid eliminated. There is, on the other hand, a great

increase in the ammonia, which bears the brunt of the burden, and usually a very definite increase in the titratable acidity of the urine. The relation of the total calcium output in the urine to the sum of the ammonia and titratable acidity, in the various observations on our first three patients is represented in figure 3. From a glance at this chart it is obvious that the proportion of calcium to the ammonia and titratable acidity is small. Even in acidosis when, as in DA, (column D), the plasma CO<sub>2</sub> combining power had fallen to 32 volumes per cent, the greatly increased calcium output of the urine was relatively insignificant when compared with the extent of the ammonia and titratable acidity. Moreover, in our cases, (e.g., BE Case IV, periods IX-XI, XIV) the increase in ammonia and titratable acidity in response to ingestion of NH<sub>4</sub>Cl soon reached a value that was almost equal to that of the total excess acid ingested.

Even if all the calcium excreted could act as alkali, its rôle in neutralizing acid would be relatively insignificant. It is probable, however, that some of the extra calcium, when excreted, is accompanied by acid radicles with which it was associated in the body stores whence it originated, whether from soft tissue or from bone. This consideration would tend further to minimize the availability of calcium as a reserve alkali.

It may further be pointed out that when a neutral diet of higher calcium content was taken with the simultaneous ingestion of 6 grams of NH<sub>4</sub>Cl per day, as in BE (Case IV, periods XV-XVII) the greatly increased amount of calcium in the urine failed to reduce the excretion of urinary ammonia.

Our results, showing the small part played by calcium in neutralizing excess acid of the urine, are similar to those obtained by Gamble, Ross, and Tisdall (21) in fasting children.

*The relation of the increased calcium excretion to the acidity of the urine and ammonia production*

The "total acid" excretion of AN (Case I) was unusual in that so much ammonia was produced that the urine remained alkaline in reaction even when large amounts of excess acid were ingested. In spite of the fact that the urine remained alkaline, great increases in urinary calcium excretion followed upon ingestion of acid diets and of

NH<sub>4</sub>Cl The calcium excretion, in fact, seemed to vary with the total amount of excess acid eliminated, and especially with the amount of ammonia excreted, irrespective of the reaction of the urine. This peculiar overproduction of ammonia is discussed at greater length in another paper (22).

In response to ingestion of excess acid except in the case of acid phosphate, ammonia production played a much greater rôle than did increased titratable acidity of the urine. Ammonia production increased promptly but gradually as did the calcium of the urine, remained high throughout the period of acid ingestion, and like the calcium fell off gradually in the after period. The titratable acidity on the other hand usually increased to its maximum more rapidly than did either ammonia or calcium and on discontinuing administration of excess acids it fell off abruptly to levels below those of the control period. The ingestion of acid phosphate, however, caused a great increase in titratable acidity of the urine but no appreciable effect on either ammonia or calcium (20).

It is probable that increased ammonia production and increased urinary calcium excretion are separate responses to a common cause and not directly interdependent. Both are greatest when as in the cases of DA (Case II, periods XIV and XV) and ST (Case III, periods XII and XIII) there is a definite acidosis with low plasma CO<sub>2</sub>. Neither are materially affected by ingestion of NaH<sub>2</sub>PO<sub>4</sub>, which (being readily excreted in an acid urine) places little strain on the mechanism that regulates the acid base balance.

#### *The excretion of calcium and fixed base*

In response to administration of large doses of NH<sub>4</sub>Cl (e.g. ST, Case III, periods XI-XIII) there is a prompt rise in the excretion of total fixed base in the urine, which in our cases was maximal in the first period of increased acid administration. This increase in excretion of total fixed base is associated with a rapid loss of body weight (water and electrolytes) which has been well discussed by Gamble, Blackfan and Hamilton (23). There is thus seen to be a very great difference between the response in total fixed base excretion and in that of calcium. The output of total fixed base rises rapidly with the initial diuresis and loss of weight and then falls toward its former level.

Immediately on cessation of NH<sub>4</sub>Cl the excretion of fixed base falls temporarily to much below that of the control periods while the body retains water and gains in weight. The calcium excretion, on the other hand, rises gradually in response to ingestion of acid, requiring a few days to reach its maximal level at which it remains while excess acid is administered, then falls off slowly, taking several days to return to the control level.

That the amount of calcium of lost body fluids was quite insufficient to account for extra calcium eliminated in fasting children was shown by Gamble (21). Similarly, in our cases the calcium of the body fluids lost in diuresis is insignificant when compared with total extra excretion of calcium in the urine. Moreover, as just noted, the diuresis was over before the calcium excretion reached its maximum and in the after-period there was no compensatory retention of calcium.

#### *The effect of ingestion of NH<sub>4</sub>Cl on the nitrogen metabolism*

In most of our subjects the ingestion of NH<sub>4</sub>Cl resulted in a greater increase in the nitrogen of the urine than could be accounted for by the increased intake. This was associated with an increase in excretion of inorganic sulphate. In the after periods there was usually some nitrogen retention. This increased output of nitrogen was not always associated with a loss of body weight. It is obvious that nitrogen, administered as a potentially acid ammonium salt does not spare protein metabolism, but actually results in loss of nitrogen, probably from the deposit nitrogen of the organism.

#### *The relation of the calcium and phosphorus balances*

In all cases, when the excretion of calcium was greatly increased there was also a definitely increased output of phosphorus when an appreciable amount of calcium was retained, phosphorus was also retained. If one calculates the phosphorus equivalent of the extra calcium excretion on the basis of the proportion of calcium to phosphorus in bone (2 3 1) and adds to this figure that of the phosphorus equivalent of the extra nitrogen excretion (+ or -) derived from the proportion of nitrogen to phosphorus in protein (17 4 1) the result is near that actually found for increased phosphorus excretion. In

other words, the phosphorus balance varies with the phosphorus equivalents of the calcium and nitrogen balances (24, 25). The only notable departure from this generalization was that on administration of large amounts of acid, (e.g. DA Case II, periods XIV and XV) there was, at the time of the diuresis and loss of weight, a prompt increase in the phosphorus of the urine out of proportion to the change in the excretion of calcium and nitrogen. In the after-period there was a similar retention of phosphorus so that while the calcium excretion remained above basal levels for several days after the last dose of  $\text{NH}_4\text{Cl}$ , the phosphorus of the urine dropped promptly to less than that of the control periods. The final net balance, justifies the original statement.

#### *The effect of ingestion of excess alkali*

It was found impossible to devise a palatable low calcium diet that had a large potential alkalinity. Accordingly, varying amounts of sodium bicarbonate and sodium citrate were fed in order to observe the effect of ingestion of excess alkali on the excretion of calcium and phosphorus. In Case I the ingestion of 9 grams of  $\text{NaHCO}_3$  and 6 grams of sodium citrate per day produced no effect on the calcium content of either urine or stools. In Case II there was possibly a slight increase and in Case III a definite slight increase in urinary calcium, but no increase in the calcium of the feces, when very large amounts of alkali (12 grams of  $\text{NaHCO}_3$  and 12 grams sodium citrate daily) were taken. In no instance, however, did ingestion of excess alkali have any important effect on the calcium or phosphorus balance.

#### *Serum values*

It has been found that when blood is taken from normal, fasting individuals the serum calcium may remain remarkably constant over considerable periods of time, the serum phosphorus being slightly more variable (26). In this investigation also, there was very little change in the levels of serum calcium and phosphorus. In some patients, however, (DA, Case II, period XV, ST, Case III, period XIII), who had been for a long time on a low calcium diet, the administration of large amounts of  $\text{NH}_4\text{Cl}$  seemed to cause a definite fall in both the calcium and inorganic phosphorus of the fasting blood. The serum protein

## EFFECT OF ACID-PRODUCING SUBSTANCES

TABLE 6  
L.N.—Case V

Period*	Diet		Nitrogen			Calcium			Phosphorus			Remarks	
	Fat	Carbo hydrate	Protein	Respira- tory quotient		Urine	Feces	Intake	Urine	Feces	Intake		
				grams	grams								
I	129	51	32	0.77	16.65	1.67	15.1	0.13	0.76	1.02	0.13	1.36	
II	117	41	32	0.72	14.94	0.94	15.4	0.63	0.65	1.23	-0.05	1.64	
III	151	28	33	0.75	11.94	1.11	15.8	0.69	0.56	1.05	-0.20	1.44	
IV	156	20	32	0.71	13.12	1.72	15.4	0.69	0.67	0.96	-0.40	1.27	
												0.30	
												1.27	
												-0.30	

\* The values in this table represent three day averages (to be consistent with those of the preceding tables) although each period in this case actually consisted of five days.

changed relatively little but the CO<sub>2</sub> content of the plasma fell definitely after ingestion of 4 to 6 grams of NH<sub>4</sub>Cl and markedly after ingestion of larger amounts of NH<sub>4</sub>Cl. It is to be noted that the great increase in calcium excretion, which occurred on ingestion of large amounts of NH<sub>4</sub>Cl, was not associated with an increase in the serum calcium level.

#### *Effect of ketogenic diet*

The calcium and phosphorus excretion was followed in LN (Case V), a child of 7 years of age, who was given a Ketogenic diet for the treatment of epilepsy by Dr Harold Higgins. The diet throughout was approximately neutral and was well taken by the child, who was happy and cooperated willingly. Results are presented in table 6.

As the ratio of fat to carbohydrate and protein in the diet was increased, there developed a marked ketosis and an initial loss of weight (due to loss of body water). With this acidosis there was an increase in the urine calcium excretion so that the calcium balance changed from being definitely positive to distinctly negative. The phosphorus balance also became negative. These results are similar to those reported by Nelson (13) and by Sawyer, Baumann and Stevens (14).

#### *The effect of ingestion of excess acid when the serum calcium is abnormally low*

All the results discussed so far were obtained on subjects believed to have an essentially normal calcium metabolism. In the course of our investigation there arose an opportunity of studying the effect of similar measures in a patient with an abnormally low serum calcium. Mrs DB, age 27, weight 43 kilos, had suffered for four years from tetany associated with deranged fat absorption. Her history and the detailed results of the investigation are described in another paper (27).

In table 7 are presented data showing the average values for groups of periods in which she was given first a low calcium, then a higher calcium diet, and, while on the higher calcium diet, 4 grams NH<sub>4</sub>Cl, 6 grams NH<sub>4</sub>Cl, and 9 grams CaCl<sub>2</sub>, daily, respectively. On the low calcium diet the calcium excretion in the urine was exceedingly small—so little in fact that it could scarcely be measured, nor did it rise on

## EFFECT OF ACID-PRODUCING SUBSTANCES

TABLE 7  
*DB—Acute effect of ingestion of NH<sub>4</sub>Cl and CaCl<sub>2</sub> in a case of steatorrhea with tetany\**

Period	Diet	Total acid excretion				Calcium				Phosphorus				Serum values			
		Titrate cc N/10	Ammonia in urine cc N/10	Urine		Feces grams	Balance grams	Urine		Feces grams	Balance grams	Urine per 100 cc	Feces per 100 cc	Plasma protein per cent	Plasma CO <sub>2</sub> content per cent		
				Feces grams	Urine grams			Feces grams	Urine grams								
I-IV	C <sub>1</sub> 80 mgm, P 155 mgm, daily Neutral	0.012	0.708	—0.479	1.45	0.71	—0.76	5.4	2.8	6.31	59.2						
VII-XI	C <sub>1</sub> 521 mgm, P 816 mgm, daily Potentially neutral	2.351	0.009	1.254	0.280	1.84	0.64	—0.02	6.0	2.5							
VII-XV	Same plus 4 grams NH <sub>4</sub> Cl daily	3.582	0.015	1.770	—0.222	2.37	0.53	—0.45	6.0	3.0	60.0						
XVI-XVIII	Same plus 6 grams NH <sub>4</sub> Cl daily	4.072	0.017	1.970	—0.384	2.37	0.42	—0.33	7.6	2.6	58.2						
XIX-XXIV	Same—No medication	—1.44	1.890	0.015	1.750	—0.132	1.92	0.44	—0.06	7.1	3.2	6.36	59.0				
XIX-XXIV	Same plus 9 grams CaCl <sub>2</sub> daily	3.297	0.015	4.210	7.119	1.96	0.66	—0.23	7.2	2.4	6.60	57.7					

\*The full table will be published in Paper VI of this series (27).

ingestion of higher calcium diet,  $\text{NH}_4\text{Cl}$  or  $\text{CaCl}_2$ . The fecal calcium on the other hand was unusually high so that for most of the time there was a negative calcium balance. The proportion of phosphorus excreted in the urine was within normal limits. Associated with the negative calcium balance there was a negative phosphorus balance.

Although ingestion of potential acid resulted in great increase in ammonia production, yet in contrast to those cases with normal serum calcium the urinary calcium output was not increased even when the serum calcium rose from about 5 to about 7 mgm there was no change in the urinary excretion. That the low calcium excretion of this patient was not entirely dependent on the low serum calcium is shown by observations previously made (27) on a case of postoperative tetany, in which administration of  $\text{NH}_4\text{Cl}$  resulted in a distinct increase of calcium in the urine. In the postoperative patient, however, the initial urinary calcium was much higher and presumably body stores were much greater. In our patient the factors limiting urinary calcium excretion were such that the calcium excretion was too little to be measured with accuracy.

#### SUMMARY

The effect of large variation in the acid base balance as brought about by ingestion of ammonium chloride, sodium bicarbonate and sodium citrate and of potentially acid and ketogenic diets, has been studied in a number of patients on a fixed low-calcium diet and in one patient receiving a higher calcium diet.

The calcium excretion was found to vary with the total excess acid eliminated and more particularly with the output of ammonia, and appeared to be independent of the reaction of the urine.

The quantitative increase in calcium excretion in response to ingestion of acid was greatly influenced by the basal level of calcium excretion as well as by the amount of excess acid ingested.

The increased calcium excreted played a very small rôle in balancing excess acid output, even though the calcium of the urine was increased many times above the basal level.

Calcium elimination was only slightly affected by large doses of alkali.

On low-calcium diet the fecal calcium remained nearly constant despite great changes in the potential acidity of the diet

In one patient with steatorrheic tetany and persistently low serum calcium, administration of higher calcium diet,  $\text{NH}_4\text{Cl}$  and  $\text{CaCl}_2$ , failed to cause an increased output of calcium in the urine

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## STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM

### XIII THE EFFECT OF INGESTION OF PHOSPHATES ON THE EXCRETION OF CALCIUM<sup>1</sup>

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Most of the calcium of the body is found combined with phosphate in the skeleton but phosphorus in one form or another is present in relatively large quantities in all body tissue. Any gross change in calcium metabolism must of necessity affect the phosphorus output. It might be possible, however, for considerable changes to occur in phosphorus metabolism without any great effect on the calcium balance.

In the metabolism of bone the two elements are closely associated and under certain abnormal conditions their mutual interdependence is strikingly demonstrated. It has been clearly shown, for instance, (1, 2, 3, 4, 5, 6) that in growing animals the ratio of these two elements in the diet is of the greatest importance and that in the absence of the controlling influence of vitamin D any great departure from the optimal relationship of calcium and phosphorus in the food results in the development of rickets. In the rachitic child or rat, also, Karelitz and Shohl (7) and Shohl and Brown (8) found that the ingestion of added phosphate brought on tetany with the characteristic fall in serum calcium and rise in serum phosphorus.

It is well known that in many conditions there is a definite relationship between the values for calcium and phosphorus in the serum.<sup>3</sup> In

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<sup>3</sup> The term "serum phosphorus" is used with reference to serum inorganic phosphorus throughout this paper.

tetany, whether infantile, idiopathic, or after parathyroidectomy, the low serum calcium is almost always associated with a high serum phosphorus. A similar relationship was found to exist in terminal nephritis by Marriott and Howland (9), DeWesselow (10), and others. In hyperparathyroidism, on the other hand, whether induced by injections of parathyroid extract or present as a pathological state (11, 12, 13, 14, 15), the serum calcium is high and the serum phosphorus low. In those conditions characterized by low serum calcium and high serum phosphorus the excretion of calcium in the urine tends to be diminished. When, on the other hand, the serum calcium is high and serum phosphorus low, the excretion of both in the urine is increased.

In exophthalmic goiter, Aub, Bauer, Heath, and Ropes (16) found associated with a greatly increased urinary excretion of calcium and nitrogen a corresponding increase in the phosphorus excretion. The converse was found to be true of myxedema. In neither case were serum levels changed appreciably.

It was first observed by Binger (17) that in dogs the injection of large amounts of neutral phosphate caused a marked lowering of the serum calcium with the development of tetany. Tisdall (18) obtained similar results and Salvesen, Hastings, and McIntosh (19) found that ingestion of very large quantities of inorganic phosphate, whether acid or basic, had the same effect. Injection of phosphate in dogs was found by Greenwald and Gross (20) to cause an increased excretion of calcium which persisted for several days. Ingestion of excess phosphorus in children has also been found to result in an increased loss of calcium in the feces (21).

Although such close relationship has been found in the metabolism of calcium and phosphorus, no careful studies have been made of the influence of changes in the phosphorus intake on the calcium metabolism of human adults. The effects noted above, of injection or ingestion of phosphate, were produced in dogs by tremendous quantities which were far in excess of any physiological variation in the phosphorus intake. The purpose of the present investigation was to study the effect of great variations in the ingestion of phosphorus on the calcium balance of adults who had no disorder likely to affect the inorganic salt metabolism. Experiments were planned to differentiate clearly the influence of the phosphate radicle itself from that of the

acid or basic properties of its various salts as well as from any possible effect of an increased nitrogen metabolism when the phosphorus intake was increased by feeding a high protein diet.

#### EXPERIMENTAL PROCEDURE

Most of the patients were given a control diet low in calcium and phosphorus but adequate with respect to other inorganic salts, vitamins, fat, carbohydrate, protein, and total caloric content, as described in a former paper (22). This control diet was so constituted as to give a neutral inorganic residuum when oxidized. When there was of necessity an excess intake of acid radicles, as in a high protein diet, the acid effect was controlled by the administration of an appropriate amount of sodium bicarbonate with each meal. Urine and stools were collected and prepared in three-day periods, as formerly described (22), the specimens for the first three days being discarded. Whenever possible a further nine days was allowed for an initial observation period, nine days for testing each change in regimen, and an after period of nine days for return to equilibrium before another change was instituted.

#### METHODS

Calcium determinations were made by Fiske's method (23). This method has been found to be thoroughly dependable and more accurate than methods previously used, especially under conditions when large amounts of phosphate are excreted. Inorganic phosphorus, urinary ammonia, titratable acidity of the urine, and total base, were done by the methods of Fiske and Subbarow, Folin, Henderson and Palmer, and Fiske, respectively, under conditions or minor alterations as previously described (22).

#### EXPERIMENTS AND RESULTS

##### *The effect of large doses of acid sodium phosphate*

WN, a married woman of thirty four years of age, suffering from chronic atrophic arthritis, was given throughout the whole period of observation the same basal diet low in calcium and phosphorus, and potentially neutral. After a nine-day control period 15 grams of  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  was given daily for eight days. Observations were

## EFFECT OF INGESTION OF PHOSPHATES

TABLE I  
Case IVN The effect of acid phosphate administration

Diet	Period	Case IVN				Calcium				Phosphorus			
		Total acid excretion		Nitrogen		Output		Intake		Output		Intake	
		Titratable acidity Y/10	Amonia in urine	Output in urine	Intake	Urine	Feces	Total	Balance	Urine	Feces	Total	Balance
Control	I	193*	550*	743*	21.7	27.5	0.21	0.39	0.63	0.29	-0.34	1.13	0.53
	II	192	556	718	22.0	27.8	0.33	0.16	0.79	0.28	-0.51	1.26	0.56
	III									-0.64	1.35	0.64	1.99
$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	IV	352	597	949	21.9	28.2	0.36	0.49	0.85	0.28	-0.59	2.18	0.62
	V	1,222	758	1,980	20.0	27.8	0.26	0.55	0.81	0.28	-0.53	7.33	1.62
	VI	870	776	1,616	19.9	27.8	0.33	0.11	0.74	0.28	-0.16	6.77	2.06
Control	VII†	182	956	1,338	14.6	19.0	0.24	0.47	0.71	0.19	-0.52	1.57	0.89
	VIII	142	914	1,056	17.1	26.1	0.20	0.61	0.81	0.27	-0.54	0.78	0.70
	IX	362	932	1,294	20.5	27.9	0.33	0.67	1.00	0.28	-0.72	1.26	0.76

\* Calculated from one day's results only.

† In period VII patient suffered from an acute upper respiratory infection.

Period IV, 10 grm. of  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  ingested on last day. Periods V and VI, 15 grms of  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  were ingested daily.

A constant neutral low calcium diet was eaten daily throughout the observation.

continued for another nine day after-period. Early in the after period the patient had an acute upper respiratory infection with a fever of 102°. This condition, however, subsided quickly without having any obvious effect on the excretion of either calcium or phosphorus. It had previously been shown that chronic fevers (16) failed to alter materially the calcium and phosphorus metabolism. The results are given in table 1.

It is quite clear that the ingestion of acid sodium phosphate, in such quantity that the phosphorus intake was increased to more than five times that of the control diet, had no appreciable effect on the excretion of calcium in either urine or feces. The average negative calcium balances for the initial control periods, the periods of administration of acid phosphate, and the final control periods were -0.50, -0.53, and -0.60 grams respectively. It is to be noted also that the increase in the titratable acidity of the urine in response to the ingestion of acid phosphate was much greater than that of the urinary ammonia. Even the slight increase in the ammonia excretion that occurred in this patient on the ingestion of acid phosphate is unusual. It is difficult to explain the further rise in ammonia excretion during the after-period, unless it be in some way associated with the acute infection from which the patient suffered in period VII. It is obviously not related to an increased phosphorus excretion.

*Comparison of effects of ingestion of equal amounts of phosphorus as acid and as basic phosphate*

In order to distinguish between the effect of the phosphate radicle and that of the acid or basic qualities of its salts, a patient, while maintained on a constant control diet low in calcium and potentially neutral, was given at appropriate intervals about 2.3 grams of phosphorus daily, first as  $\text{Na}_2\text{HPO}_4$  and then as  $\text{NaH}_2\text{PO}_4$ . RN, a woman of thirty nine years of age, weighing fifty-six kilos, had suffered from rheumatic heart disease (mitral stenosis), chronic bronchitis, and bronchial asthma. During the period of observation, and for some time before, however, she had no cough, her chest was clear, and there were no signs of myocardial failure. She was comfortable and looked well. In period X, while receiving acid phosphate, she had some

## EFFECT OF INGESTION OF PHOSPHATES

TABLE 2  
Cate RN. The effect of phosphate administration

Diet	Period	Total acid excretion				Nitrogen				Calcium				Phosphorus			
		Titer table acidity of urine	Am monia in urine	Output in urine	Intake	Output			Intake	Intake			Output			Intake	
						grams	grams	grams		Feces	Urine	Total	grams	grams	grams	grams	grams
Control	I	cc N/10	cc N/10	24.1	25.0	0.16	0.21	0.37	0.29	-0.08	1.10	0.50	1.90	1.81	-0.09		
	II	531	692	1,223	23.8	0.11	0.34	0.15	0.29	-0.16	1.33	0.65	1.98	1.81	-0.17		
	III	115	598	1,013	23.0	0.10	0.41	0.51	0.29	-0.22	1.31	0.63	1.91	1.81	-0.13		
	IV	526	635	1,161	22.2	15.1	0.14	0.26	0.10	0.18	-0.22	1.36	0.59	1.95	1.16	-0.79	
Control + NaH <sub>2</sub> PO <sub>4</sub>	V	70	306	376	16.6	25.0	0.10	0.32	0.42	0.29	-0.14	3.16	3.33	6.19	8.45	1.96	
	VI	-397	313	-84	22.0	22.7	0.10	0.42	0.52	0.25	-0.27	5.32	1.94	7.26	8.60	1.40	
	VII	17	276	293	17.7	18.2	0.13	0.41	0.54	0.23	-0.31	5.00	1.28	6.28	8.10	2.10	
	VIII	106	116	722	16.6	20.5	0.13	0.39	0.52	0.26	-0.26	1.62	0.79	2.11	1.57	-0.81	
Control + NaH <sub>2</sub> PO <sub>4</sub>	IX	180	127	607	18.0	24.5	0.19	0.41	0.60	0.29	-0.31	0.86	0.65	1.51	1.97	0.36	
	X	1,008	697	1,705	19.1	25.9	0.14	0.44	0.58	0.30	-0.28	8.83	2.59	7.12	9.01	1.59	
	XI	978	572	1,450	17.5	23.8	0.11	0.30	0.41	0.27	-0.17	5.44	1.32	6.76	8.87	2.11	
	XII	520	584	1,104	19.9	24.8	0.27	0.42	0.69	0.29	-0.40	2.54	0.75	3.29	1.78	-1.51	
Control	XIII	412	639	1,051	17.8	23.5	0.12	0.26	0.46	0.15	-0.22	1.15	1.57				
	XIV	370	611	981	16.8	25.0	0.16	0.40	0.56	0.30	-0.26	0.97	0.66	1.63	1.81	0.15	

\* Mild diarrhea in period X.

† constant neutral low calcium diet was eaten daily throughout the observation periods V, VI, and VII, an average of 10 grams of NaH<sub>2</sub>PO<sub>4</sub> were ingested daily. Periods X and XI, an average of 12 grams of NaH<sub>2</sub>PO<sub>4</sub> were ingested daily. These represent essentially equivalent amounts of phosphorus.

diarrhea but it ceased after a day or two. Results are presented in table 2.

Again, it can be seen that the ingestion of large doses of phosphate, acid or basic, had no appreciable effect on the excretion of calcium in either urine or stool. When the disodium salt was fed the urine became alkaline and the ammonia excretion fell off but the excretion of calcium remained within the limits found in the initial control periods. On administration of acid phosphate the titratable acidity of the urine rose greatly while the ammonia and calcium excretion remained at basal levels. In both cases, even when there was diarrhea, the great bulk of the phosphate was absorbed and the urinary phosphorus was always much greater than the fecal phosphorus. There was also a marked retention of phosphorus.

#### *The acid effects of $\text{NaH}_2\text{PO}_4$ .*

In studying the influence of ingestion of various types of acid-yielding substances on the calcium and phosphorus metabolism, approximately equal amounts of "potential acid" were fed at appropriate intervals, first in the form of foodstuffs, then as  $\text{NH}_4\text{Cl}$ , and later as  $\text{NaH}_2\text{PO}_4$ , to a patient, DA, who was recovering from chronic lead poisoning. He was thirty-seven years of age, weighed thirty-eight kilos, was comfortable, and took his food well and willingly throughout the whole period of observation. The calcium intake was constant and, except during the period when an acid diet was given, the same constant neutral low calcium diet was taken throughout. Results are presented in table 3.

The sum of the ammonia excretion and titratable acidity of the urine is considered here, as in our other work, to give the best indication of the potential acid value of the ingested food or medication. In this experiment approximately equal values for this "total acid" of the urine were obtained during the ingestion of an acid diet, on administration of 4 grams of  $\text{NH}_4\text{Cl}$  daily, and on administration of 2.66 grams of phosphorus in the form of  $\text{NaH}_2\text{PO}_4$  daily. After the periods in which he received 4 grams  $\text{NH}_4\text{Cl}$  daily the dose was increased to 12 grams per day for two more periods. In response to this large amount of acid ingested there was a very great production of

TABLE 3  
Case D.A. Effect of ingestion of different types of acid (potentially acid diet,  $\text{NH}_4\text{Cl}$ , and  $\text{NaH}_2\text{PO}_4$ ) on calcium excretion

Period	Total acid excretion		Nitrogen		Calcium		Phosphorus		Treatment and remarks	
			Output		Output		Output			
	Total N/10 cc	N/10 grams	Output in urine + amm. acids N/10 cc	Urine N/10 grams	Total urine grams	Urine grams	Balance urine grams	Urine grams	Total urine grams	Urine grams
III*	134	563	997	22.6	29.3	0.09	0.53	0.02	0.33	-0.29
VI	509	1,350	1,867	24.3	49.0	0.18	0.50	0.68	0.30	-0.38
VII	667	1,631	2,298	28.2	32.2	0.18	0.48	1.66	0.34	-0.32
VIII	870	1,797	5,657	36.7	38.7	0.73	0.53	1.26	0.35	-0.91
XI	259	1,100	3,659	28.0	29.3	0.16	0.83	0.99	0.35	-0.64
XII	60	2,543	2,603	21.0	20.3	0.15	0.45	0.60	0.35	-0.25
XIII	310	1,562	1,872	15.7	20.3	0.23	0.46	0.69	0.35	-0.34

XIX	1,213	1,362	349	21	5	29	3	0	07	0	33	0	41	0	33	-0	08	4	57	3	01	7	58	10	14	2	56
XX	1,179	699	1,878	21	5	29	3	0	06	0	56	0	62	0	34	-0	28	5	91	3	82	9	73	10	14	0	41
XXI	295	450	745	21	6	29	3	0	10	0	50	0	60	0	34	-0	26	2	31	1	57	3	88	3	04	-0	84
XXII	274	444	718	19	3	29	3	0	07	0	48	0	56	0	35	-0	21	0	86	1	05	1	91	2	15	0	24
XXIII	245	510	755	20	7	29	3	0	07	0	45	0	53	0	35	-0	18	0	69	1	00	1	69	2	15	0	46

\* The full tables are given in Paper XII (28)

ammonia,<sup>4</sup> and this high level of ammonia excretion persisted on into the after-control period and unfortunately had not yet reached a steady state when the acid phosphate was given nine days later. The nature of the response to  $\text{NaH}_2\text{PO}_4$  is, nevertheless clear.

The different types of acid administered provoked a distinctly different response as reflected in the nature of the increased acid in the urine. It has been pointed out elsewhere (24) that although the total acid output of the urine was approximately equal for the three types of acid ingested, nevertheless, when the acid was given as excess acid in the food or as  $\text{NH}_4\text{Cl}$ , the brunt of the burden was borne by increased ammonia production, and the titratable acidity of the urine was only slightly increased. When acid phosphate was given, however, the titratable acidity increased greatly and the ammonia excretion, still high after the ingestion of  $\text{NH}_4\text{Cl}$ , continued to fall while acid-phosphate was taken. In the second phosphate period it was only slightly higher than that of the control periods.

There was likewise a very great difference in the effect of the different types of acids on the calcium excretion. Those whose ingestion was associated with great ammonia production also gave rise to a considerable increase in the calcium excretion in the urine. The ingestion of  $\text{NaH}_2\text{PO}_4$ , on the other hand, which produced only an increased titratable acidity, was associated not with increased calcium excretion but actually with a slightly diminished output of calcium in the urine. The values for calcium in the feces were little affected by the different types of acid fed, although like those for the urine the lowest values for calcium in the feces of the whole period of observation were obtained during the administration of  $\text{NaH}_2\text{PO}_4$ .

The results of these three experiments show that the ingestion of large amounts of inorganic phosphate, whether acid or basic, fails to influence appreciably the excretion of calcium in either urine or feces in adults on a low calcium diet. The intake of phosphorus in these instances was much greater than could be fed in any diet of natural foodstuffs, corresponding as it did to the phosphorus content of a diet containing well over 200 grams of protein.

<sup>4</sup> That the increased excretion of ammonia which follows on ingestion of  $\text{NH}_4\text{Cl}$  is not due to an increased intake of "ammonia" has been clearly shown in another paper (24). The great excretion of ammonia fell to control levels when an appropriate amount of  $\text{NaHCO}_3$  was ingested between doses of  $\text{NH}_4\text{Cl}$ .

*Effect of food rich in phosphorus*

In hyperthyroidism (16) it had been found that a greatly increased excretion of calcium and nitrogen was associated with a corresponding increase in the phosphorus output. To ascertain whether a diet high in protein (i.e. high in phosphorus) would affect the calcium metabolism, 200 grams of protein per day were fed to a healthy normal individual, aged thirty, weighing seventy-eight kilos. In the previous nine days the subject had taken a neutral control diet of approximately the same low calcium content as that of the high protein diet. During the first nine days of the high protein diet sufficient  $\text{NaHCO}_3$  was taken with the meals to prevent an increase in the "total acid" excretion of the urine, thus eliminating the acid effect of the high protein diet. Results are given in table 4.

On the ingestion of large quantities of protein there was a gradual but definite increase in the calcium excreted in the urine associated with an almost equal decrease in the calcium of the feces, so that the calcium balance for the period of high protein diet plus  $\text{NaHCO}_3$  was virtually the same as for the control period. The high protein diet had a low residue and during its administration the stools were smaller than formerly although there was no tendency toward constipation. The lower calcium of the stool may be accounted for, in part at least, by the smaller bulk of the feces, as suggested by observations of Sjollema (25). The phosphate of the feces was little increased and after  $\text{NaHCO}_3$  was discontinued it was actually slightly less than that of the control periods. Phosphorus in the urine increased to over 4 grams, and even on such high intake there was a slight negative phosphorus balance.

The very high protein diet had no appreciable effect on the calcium balance so long as  $\text{NaHCO}_3$  was taken to neutralize its acid effect. When the  $\text{NaHCO}_3$  was discontinued the ammonia, titratable acidity, and calcium of the urine all rose but the experiment had to be discontinued before the full effect could be observed.

*Moderate calcium diet—Effect of ingestion of added phosphate*

All of the previous experiments had been done on patients taking a low calcium diet. The effect of equal amounts of phosphate, first in a

TABLE 4  
Case R F F Effect of high protein diet on calcium metabolism

Diet	Period	Total acid excretion						Calcium						Phosphorus					
		Nitrogen			Output			In take			Output			Intake			Balance		
		Titratable acidity of urine	Amonia monia in urine	NaHCO <sub>3</sub> added	Urine	In take	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	
Control	I	521	931	1,455	32.0	28.0	0.29	0.58	0.87	0.34	-0.53	2.11	0.70	2.81	2.08	-0.73			
	II	290	854	1,114	27.0	28.0	0.32	0.58	0.90	0.34	-0.56	1.93	0.69	2.62	2.05	-0.57			
	III	259	750	1,009	24.0	28.0	0.26	0.55	0.81	0.34	-0.17	1.81	0.66	2.17	2.08	-0.39			
	IV	-265	984	719	2,500	70.0	0.96	0.38	0.54	0.92	0.36	-0.56	3.85	0.74	4.59	4.71	0.12		
	V	-28	928	900	3,150	83.2	0.96	0.37	0.54	0.91	0.36	-0.55	4.11	0.93	5.04	4.71	-0.33		
	VI	50	1,088	1,138	3,030	89.0	0.96	0.15	0.38	0.83	0.36	-0.18	4.11	0.81	5.25	4.71	-0.51		
High protein + NaHCO <sub>3</sub>	VII	238*	181*	594*	2,926	83.1	0.91	0.16	0.32	0.78	0.35	-0.13	4.13	0.62	5.05	4.60	-0.15		
	VIII	106*	192*	716*	778*								0.55		5.22				

#### Daily determination

Periods I-III, control low calcium diet, 58 grams protein Periods IV-VI, low calcium diet, 202 grams protein plus NaHCO<sub>3</sub>, to control acidity of protein diet Periods VII and VIII, low calcium, high protein diet only

high protein diet and then as added (nearly) neutral inorganic phosphate, was now studied on a patient receiving a moderate calcium intake of about 0.5 gram per day instead of 0.1 gram previously given LZ, aged eighteen years, weighing sixty kilos, was slowly recovering from chronic multiple neuritis. He was confined to bed throughout the whole period of observation but looked and felt fairly well. After two periods on a diet containing 0.50 gram calcium and 66 grams protein per day he was given a diet of the same calcium content but containing about 120 grams of protein per day.  $\text{NaHCO}_3$  was added to control the acidity. Later, after an interval of nine days on the control diet, he was given approximately the same amount of phosphorus in an equimolecular mixture of  $\text{NaH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$ . Results are presented in table 5.

The calcium excretion in the urine of this patient was higher than is usual. On an intake of 0.50 gram per day there was in the control periods an average negative calcium balance of 0.44 gram per three-day period. He was kept on the high protein diet for eighteen days, during all of which time he was given  $\text{NaHCO}_3$ . The average value for urinary calcium of the high protein periods is almost exactly the same as that for the preceding and for the following control periods. During the high protein diet, however, the stools were very much larger than in the control periods, and their calcium content was definitely, although not greatly, increased. There was, accordingly, a greater negative calcium balance during the period of high protein diet. This finding is quite the reverse of that obtained in subject R. F. F. on a high protein diet. In R. F. F., however, during the ingestion of the high protein diet the stools were smaller than in the control periods. It seems reasonable to attribute the larger output of calcium in the stools of LZ not to any specific effect of increased ingestion of protein and phosphorus but rather to associate it with the greater fecal bulk (due to other causes).

The subsequent administration of 1.1 gram of phosphorus as an equimolecular mixture of  $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$  resulted in a slight decrease in ammonia and a definite decrease in the calcium of the urine. The size and calcium content of the stools was rather variable but it is clear the administration of inorganic phosphate failed to cause an increase in fecal calcium excretion. The excretion of phosphorus

TABLE 5  
Case LZ Effect of high protein diet and of nearly neutral phosphate

Diet	Period	Nitrogen		Calcium		Phosphorus		Balance grams	
		Output		Intake		Intake			
		Output grams	Excess grams	Total grams	Intake grams	Total grams	Intake grams		
Control		NH <sub>4</sub> NO <sub>3</sub> cc g/10	NH <sub>4</sub> NO <sub>3</sub> cc g/10	Output in urine grams	Intake grams	Intake grams	Intake grams	Balance grams	
	I	40	579	619	28.5	39.7	1.04	-0.32	
	II	94	562	656	30.0	31.7	1.01	-0.35	
	III	279	826	1,105*	45.6	76.5	0.95	-0.45	
	IV	148	889	1,037	52.8	62.7	1.20	-0.16	
	V	-591	538	-53	46.1	56.6	0.80	-0.15	
	VI	390	1,035	1,425	47.0	56.6	0.98	-0.09	
	VII	-57	814	757	2,142	44.0	48.2	-0.87	
	VIII	-330	738	408	2,142	47.3	53.1	-0.83	
	IX	-169	652	483	33.9	32.8	1.00	-0.83	
	X	-121	608	487	28.5	31.5	1.12	-0.83	
	XI	-4	609	605	27.4	31.7	0.91	-0.82	
Control								Balance grams	
								2.38	
								0.60	
								2.98	
								2.40	
								-0.58	
								-1.16	
								-1.02	
Control + mor-	XII	-263	541	278	24.1	31.7	0.86	0.10	
ganic phos-	XIII	113	525	638	20.2	30.7	0.63	0.10	
phate	XIV	70	362	432	16.7	31.7	0.42	0.02	
Control	XV	-579	406	-173	18.4	31.7	0.76	-0.24	
	XVI	-241	507	266	20.0	31.7	0.85	-0.29	

\* These varied with the amounts of NaHCO<sub>3</sub> ingested.

in both urine and feces was higher on the high protein diet than when the same amount of phosphorus was fed as inorganic phosphate

In this patient receiving a moderate calcium intake the ingestion of large amounts of phosphorus either in protein or as inorganic phosphorus had produced no great effect on the calcium balance. In his case, however, as in the case of DA, the taking of large amounts of inorganic phosphate was associated with a definite but not great fall in the calcium output in the urine.

#### *Effect of ingestion of phosphate on the serum calcium*

In the various experiments blood was taken during fasting just before making a change in phosphorus intake. Under such conditions there was at no time an appreciable change in the serum calcium except that in the case of DA the serum calcium was slightly lowered after ingestion of large doses of  $\text{NaH}_2\text{PO}_4$ .

In one subject blood determinations were made at frequent intervals after a single dose of 3 grams of phosphorus as nearly neutral phosphate. Although the serum phosphate rose from 3.4 mgm to 5.0 mgm the serum calcium values were not affected. These results are discussed more fully in another paper (26).

#### COMMENT

These metabolic studies, with one exception, were made on adults given a diet which was neutral and adequate except in calcium. Wide variation in the ratio of phosphorus to calcium in the intake appears to have but little effect on the excretion of calcium in either urine or feces, or on the calcium balance. Even when the phosphorus intake was increased fivefold the only effect noted was a slight lowering of the calcium of the urine in one case. In the patient studied on a higher calcium intake, the effect of more than doubling the phosphorus intake was similarly negative.

When the acidity of a high protein diet was controlled by the addition of  $\text{NaHCO}_3$ , there was likewise no appreciable effect of such high phosphorus and protein intake on the calcium balance in patients on low or moderate calcium intakes.

The amounts of inorganic phosphorus fed were in general the largest that could be taken without the development of diarrhea. In all

## EFFECT OF INGESTION OF PHOSPHATES

Phosphorus	Output	Intralve			Balance grams
		Feces grams	Urine grams	Total grams	
1.80	0.61	2.41	2.09	-0.32	
2.08	0.36	2.44	2.09	-0.35	
4.02	0.83	4.85	7.62	2.77	
1.61	2.48	7.09	6.15	-0.94	
1.15	1.69	5.84	5.66	-0.18	
1.30	2.08	6.38	5.65	-0.73	
3.51	2.42	5.93	4.83	-1.10	
4.66	1.62	6.28	5.29	-0.99	
2.38	0.60	2.98	2.40	-0.58	
2.69	0.52	3.21	2.05	-1.16	
1.98	1.13	3.11	2.09	-1.02	
0.66	3.95	5.39	1.44		
	5.24	5.36	0.12		
	5.39	0.70			
			1.01		

Great variation in the phosphorus intake and, therefore, in the P/Ca ratio failed to affect materially the excretion of calcium in either urine or feces, although in two cases administration of inorganic phosphate resulted in a slight but definite decrease of the calcium of the urine.

Ingestion of a very high protein diet had no appreciable effect on the calcium balance when the acid effect of the protein was controlled by administration of NaHCO<sub>3</sub>.

Ingestion of NaH<sub>2</sub>PO<sub>4</sub> gave rise to a great increase in titratable acidity of the urine but had little effect on the excretion of ammonia or calcium. In this it differed from other acid-producing substances studied, which resulted in increasing both the ammonia and calcium of the urine.

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## STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM

### XVIII ON TEMPORARY FLUCTUATIONS IN THE LEVEL OF CALCIUM AND INORGANIC PHOSPHORUS IN BLOOD SERUM OF NORMAL INDIVIDUALS

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In the investigation of various problems of calcium and phosphorus metabolism, it has been found that great changes in the volume and direction of the calcium and phosphorus stream may occur without any notable difference in the serum levels of these elements. In hyperthyroidism the calcium excretion in the urine is relatively huge and there are large negative balances of calcium and phosphorus, yet the serum calcium and serum phosphorus<sup>1</sup> remain within normal limits (1). Moreover, there is no appreciable change in the serum levels of healthy people when, by changing the calcium intake, a positive balance is shifted to a negative one or vice versa.

Yet the actual and relative amounts of calcium and phosphorus in the serum is of the utmost importance in certain abnormal conditions. MacCallum and Voegtlín (2) described the low blood and tissue calcium associated with tetany after removal of the parathyroids. Greenwald (3) later demonstrated the associated rise in serum phosphorus and since that time the high phosphorus, low calcium of the serum in many types of tetany has come to be recognized. Marriot and Howland (4), DeWesselow (5) and others have found a similar relationship of the serum values in terminal nephritis. On injection of large amounts of inorganic phosphate into dogs Binger (6) produced tetany with a low serum calcium and high serum phosphorus. Similar results have been found to follow injection or ingestion of very large quantities of phosphate by numerous investigators (7, 8, 9). The

<sup>1</sup> Throughout this paper the term "serum phosphorus" is used in referring to serum inorganic phosphorus.

feeding of much smaller amounts of phosphate to rachitic rats has been found to produce tetany with the typical blood changes by Karelitz and Shohl (10). That in rachitic animals even the high phosphorus metabolism of fasting may have similar effects has been shown by Cavins, (11), Wilder, (12), and Shohl and Brown (13). The ingestion of sodium oxalate in dogs was found to result in a low blood calcium, high phosphorus, and tetany (14).

In states of hyperparathyroidism, on the other hand, a high serum calcium is well known to be associated with a low serum phosphorus. This has been discussed in other papers (15, 16).

It is quite clear, therefore, that gross changes in the serum phosphorus value in many conditions are associated with equally marked variation of the serum calcium in the opposite direction. That the level of serum calcium is also affected by the protein content of the serum was shown by Salvesen and Linder (17). Hastings, Murray and Sendroy (18) showed that there was a linear relationship between the protein content of serum or transudate and the calcium level. Their data, taken from human sera and transudates as drawn from the body, indicate that 0.014 millimol of calcium are bound per gram of protein at the pH value of the blood. Peters and Eiserson (19) also found in a group of cases, mostly nephritics, that the concentration of calcium in the serum varies directly with the concentration of protein and inversely with the concentration of inorganic phosphorus.

Other investigators have described temporary fluctuations in either serum calcium or phosphorus of normal individuals in response to various factors. Thus, Stewart and Haldane (20) found that an increase of from 1.5 to 2 mgm in serum calcium might occur after ingestion of 30 grams  $\text{CaCl}_2$ , after forced breathing for 90 minutes, and after breathing 6 to 7 per cent  $\text{CO}_2$  for 90 minutes, and a decrease of about 2 mgm after ingestion of 60 grams  $\text{NaHCO}_3$ . Although values for serum phosphorus were not given in this paper it was reported separately (21) that forced breathing for 90 minutes resulted in every instance in a fall in serum inorganic phosphorus to below 1.25 mgm. Salvesen, Hastings, and McIntosh (22) found that intravenous injection of  $\text{CaCl}_2$  resulted in a moderate rise in serum phosphorus as well as a temporary rise in serum calcium.

The object of our investigation was to determine the extent of

temporary variation in the serum levels of calcium and phosphorus in normal adults and to what degree the inverse relationship noted above would prevail when changes in one or another component could be induced at will. Accordingly, the serum levels were determined at various times of the day in subjects on ordinary diets and after ingestion of food rich respectively in carbohydrates, fat, and protein, as well as when insulin was given preceding a carbohydrate meal. The effect of ingestion of large amounts of salts of calcium and phosphorus was also followed.

#### METHODS

Venous blood was taken from the arm into a clean syringe, previously washed with liquid paraffin, care being taken to avoid any unnecessary manipulation and to prevent hemolysis. As soon as clotting was complete the serum was separated by centrifugation, and removed. In some cases the CO<sub>2</sub> content or capacity of the plasma was determined by the Van Slyke method, the blood having been taken under oil and coagulation prevented by the use of heparin. For these determinations we are indebted to Dr A. V. Bock. Serum protein was determined by macro Kjeldahl, serum phosphorus by the method of Fiske and Subbarow (23), and serum calcium by Fiske's (24) method. The latter method has proven most satisfactory under all conditions. Determinations were done in duplicate, usually with identical results, the variation seldom being greater than 2 per cent.

In all cases, blood was first taken during fasting and then at varying intervals after different types of meals or after ingestion of calcium salts or phosphates.

#### EXPERIMENTS AND RESULTS

##### *The level during fasting*

It was important to find whether the basal fasting level would be appreciably affected by changes in type of diet or by ingestion of excess acid, alkali, or phosphate as well as to note any temporary change occurring immediately after such ingestion. Also, it was interesting to observe the relative constancy of the fasting levels of the serum calcium and phosphorus over comparatively long periods. In table 1 are presented data for such observations on two healthy individuals.

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TABLE I  
*Serum values (fasting) in two healthy subjects*

Date	Calcium	Phos phorus	Protein	Plasma $\text{CO}_2$ content	Total fixed base	Remarks
Subject R Γ Γ						
1928	<i>mgm per 100 cc</i>	<i>mgm per 100 cc</i>	<i>per cent</i>	<i>volumes per cent</i>	<i>m Eq per liter</i>	
January 27	9.6	3.4	6.9	70.7	148	Ordinary diet
February 2	9.7	4.2		71.2	152	6 grams $\text{NaHCO}_3$ taken daily, January 27 to February 2
February 6	9.6	3.6	6.3	69.5		12 grams $\text{NaHCO}_3$ + 12 grams sodium citrate February 2-6
February 9	9.1	3.2	6.4	71.8	168	52 grams $\text{NaHCO}_3$ on February 7
						56 grams $\text{NaHCO}_3$ on February 8
February 11	10.0	3.7		58.6		4 grams $\text{NH}_4\text{Cl}$ on February 9
						12 grams $\text{NH}_4\text{Cl}$ on February 10
February 13	10.0	3.4	8.0	29.1*	162	12 grams $\text{NH}_4\text{Cl}$ on February 11
February 20	9.6	3.9				15 grams $\text{NH}_4\text{Cl}$ on February 12
February 27	10.1	3.2				Ordinary diet
April 10	10.1	2.5	5.9			Ordinary diet
April 19	9.6	3.2	6.4			Low calcium diet April 5-14
April 23	10.1	3.0		63.8		High protein diet April 14-27
April 26	10.2	3.6	7.1	61.7		
June 16	9.6	3.8				High protein diet June 11-16
June 19	9.5	3.4	6.6			Ordinary diet
June 22	9.6	3.6				Ordinary diet
June 26	9.6	3.2	6.6			Ordinary diet
1930						
May 26	10.0	3.1				
Subject D M T						
1928						
February 27	10.1	3.3				Ordinary diet throughout
March 5	9.8	3.6				
June 27	9.2	3.3	6.9			
November 24	9.5	3.7				
December 10	10.0	3.5				
1930						
May 27	9.6	3.2				

\* Total  $\text{CO}_2$  oxygenated whole blood

In the case of R. F. F. the fasting serum calcium was remarkably constant at about 9.6 to 10.0 mgm per 100 cc. Under the influence of ingestion of very large amounts of alkali, it fell to 9.1 mgm, a result similar, although not as great, as that obtained by Stewart and Haldane (20). Apart from this single lower value all other determinations, including those taken during periods on very high protein diets and on ingestion of large amounts of NH<sub>4</sub>Cl, lay between 9.5 and 10.2 mgm. Variation in the serum phosphorus level was a little greater than that of calcium, ranging as it did from 2.5 to 4.2 mgm. In eight instances, when the fasting serum calcium was 9.6 or 9.7 mgm, the phosphorus varied between 4.2 and 3.2 mgm; when the serum calcium was 10.0 to 10.2 serum phosphorus values ranged from 2.5 to 3.7 mgm. The effect of high phosphorus intake in the form of a very high protein diet (200 grams protein daily) had no appreciable affect on the fasting values nor did the ingestion of large doses of NH<sub>4</sub>Cl. The serum protein varied from 5.9 to 8.0 per cent.

In subject D. M. T. a smaller number of determinations showed a slightly greater variation in the calcium and smaller variation in the phosphorus level.

That ingestion of large amounts of inorganic phosphate in patients on a low calcium diet produced no appreciable effect on the fasting serum calcium and phosphorus levels has been mentioned in another paper (25). After several weeks on a low calcium diet, also, as described elsewhere (26), ingestion of large amounts of NH<sub>4</sub>Cl was associated with slightly decreased levels for calcium and phosphorus in some cases, and ingestion of NaHCO<sub>3</sub> with increased levels for serum calcium. In all experiments, however, on ingestion of acid or alkali on high or low calcium diets, the total range of variation of many determinations for any given individual was never greater than 1 mgm calcium except in one instance. In patient D. A. (Case II, period XV), described elsewhere (26), the ingestion of large amounts of NH<sub>4</sub>Cl after the subject had been on a low calcium diet for several weeks, resulted in a fall of slightly more than 1 mgm in serum calcium.

#### *Absolute rest and mild exercise*

In one instance, it was found that there was a definite slight increase in serum calcium and protein and fall in serum phosphorus in blood

taken from a fasting, healthy subject after lying at complete rest for one and one-quarter hours. This was repeated in a number of healthy individuals, but no material change in levels of calcium or phosphorus was found, although the serum protein did change considerably. The results are presented in table 2.

TABLE 2  
*Effect of absolute rest and mild exercise during fasting*

Subject	Date	Calcium	Phos phorus	Protein	Remarks
	1928	<i>mgm per 100 cc</i>	<i>mgm per 100 cc</i>	<i>percent</i>	
R I T	April 19	{ 9.6 10.1	{ 3.2 2.8	{ 6.4 7.6	After walking $\frac{1}{2}$ mile After 1 $\frac{1}{4}$ hours complete rest
C T	June 28	{ 9.5 9.5	{ 3.8 3.5	{ 5.9 6.6	At rest, before rising After working one hour
G A	June 28	{ 9.8 9.6	{ 3.6 3.6	{ 7.1 5.9*	After working one hour After lying down one hour
D M T	November 24	{ 9.5 9.7	{ 3.7		After working three hours After lying down one hour
	December 10	{ 10.0 10.0	{ 3.5 3.2		After working three hours After lying down one hour
G P R	November 24	{ 10.4 10.4	{ 4.5		Before rising Moving about for one hour
	December 10	{ 10.2 10.4	{ 3.9 3.5		Before rising Moving about for one hour

\* Some hemolysis

#### *Fluctuations in serum calcium during the day when on ordinary diet*

The serum levels of two healthy subjects were determined at different times during a day when an ordinary diet was taken and ordinary work done. Results are presented in table 3. In subject R F F the calcium remained constant at 9.9 to 10.0 mgm, while the phosphorus varied between 3.0 to 3.8 mgm. In D M T the serum

calcium varied between 9.4 and 10.4 mgm, a range as great as that of the fasting values over many months, and the serum phosphorus between 3.1 and 3.8 mgm. It is notable that the highest value for calcium was obtained at the same time as the highest value for phosphorus.

TABLE 3  
*Serum values during the day on ordinary diet*

Subject	Date	Time	Calcium	Phosphorus	Protein	Remarks
R F F	May 26	9 00	10.0	3.1	6.8	
		9 30	Breakfast			Eggs, toast and coffee
		11 30	10.0	3.0	7.5	
		12 45	9.9	3.2	7.5	
		1 00	Lunch			Ham, carrots, potatoes, custard
		2 15	10.0	3.8	7.2	
		5 00	10.0	3.8	7.2	
		9 00	9.6	3.2	7.8	
		9 30	Breakfast			Toast, marmalade, coffee
		11 30	9.7	3.1	8.0	
D M T	May 26	12 15	Lunch			Ham potatoes carrots, custard
		1 30	9.4	3.7	7.8	
		4 45	10.4	3.8	8.3	

#### *Ingestion of carbohydrate, or carbohydrate with administration of insulin*

It has been clearly shown that the administration of insulin or the ingestion of large amounts of carbohydrate with or without insulin results in a prompt fall in the serum inorganic phosphorus (27, 28, 29). It was, therefore, interesting to find to what extent the serum calcium would vary when rapid changes in serum phosphorus were induced in this way. Accordingly, large amounts of carbohydrate in the form of ordinary foods or 100 grams of glucose were given to a number of healthy subjects and to some patients, including a case of tetany with high serum phosphorus. The changes in serum phosphorus, calcium, and blood sugar were followed for several hours. Results are given in table 4.

TABLE 4  
*Effect of ingestion of large amounts of carbohydrates*

Subject	Date	Time	Cal- cium	Phos- phorus	Blood sugar	Pro- tein	Remarks
			mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	per cent	
R T T	Febru- ary 2	Fasting	9.7	4.2			High carbohydrate break- fast
		2½ hours	9.6	3.2			
	Febru- ary 27	Fasting	10.1	3.3	81		Sugar tolerance 100 grams glucose
		½ hour	10.2	3.0	95		
		1 hour	10.3	3.0	68		
		2 hours	9.9	3.1	72		
		4 hours	10.0	3.4	68		
D M T	March 5	Fasting	9.8	3.6	86		High carbohydrate meal Carbohydrate, 122 grams Protein, 4 grams Fat, 10 grams Calcium, 64 mgm Phosphorus, 77 mgm
		½ hour	9.8	3.0	94		
		1 hour	9.8	2.9	97		
		2 hours	9.7	2.7	91		
		4 hours	9.7	3.0	83		
	March 5	Fasting	9.1	3.8	93		High carbohydrate meal Carbohydrate, 122 grams Protein, 4 grams Fat, 10 grams Calcium, 64 mgm Phosphorus, 77 mgm
		½ hour	9.1	2.8	93		
		2 hours	9.4	2.7	101		
E R		3½ hours	9.1	3.1	84		
February 9 (Tetany)	Fasting	4.7	7.5	90	7.1	High carbohydrate break- fast Carbohydrate, 171 grams Calcium, 23 mgm Phosphorus, 105 mgm	
	½ hour	4.8	7.1	124			
	1½ hours	4.4	7.1*	130			
	2 hours	4.7	6.5	110			
	3½ hours	4.2	6.6*	123			
B W	February 26 (Acromegaly)	Fasting	9.5	5.7	114	7.1	Sugar tolerance 100 grams glucose
		½ hour		4.7	147		
		2½ hours	9.7	4.7	162		
Bln	March 5 (Acromegaly)	Fasting	9.8	4.3	95		Sugar tolerance 100 grams glucose
		½ hour	9.8	4.2	143		
		1 hour	9.6	3.8	170		
		2 hours	9.8	3.7	154		
		4 hours	9.6	3.8	87		
S A	January 9 (Hypertrichosis)	Fasting	9.5	2.9	92	6.2	Sugar tolerance 100 grams glucose
		½ hour	9.5	2.5	173		
		1½ hours	10.3	2.6	150		
		3 hours	9.7	2.6	114		

\* Some hemolysis

The effects of ingestion of large amounts of carbohydrate immediately after the administration of a large dose of insulin in three normal subjects are shown by data collected in table 5

TABLE 5

*Effect of ingestion of large amounts of carbohydrate and administration of insulin*

Subject	Date	Time	Calcium	Phosphorus	Blood sugar	Protein	Remarks
			mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.		
R F F	June 26	1928					
		Fasting	9.6	3.2	98	6.6	30 units insulin
		50 minutes	9.4	2.4	105	6.9	Breakfast
		1½ hours	10.0	2.1	64	6.7	Carbohydrate, 126 grams
		3 hours	10.4	2.1	46	6.8	Protein, 8 grams
		10 grams cane sugar with tem-					Fat, 3 grams
		porary relief					Calcium, 67 mgm.
		4 hours	9.5	2.4	46	7.1	Phosphorus, 170 mgm
		30 grams glucose					
		5½ hours	9.6	2.9	46	7.2	
D M T	June 27	30 grams glucose plus 25 grams					
		bread					
		8 hours	9.7	4.2	91		
		Fasting	9.2	3.3	84	6.9	20 units insulin
		½ hour	9.6	2.8	77	7.4	Breakfast
		2 hours	9.6	2.5	73	6.8	Carbohydrate 109 grams
		3½ hours	9.8	3.0	41	6.1	Protein, 8 grams
E. R.	June 27	Lunch					Fat, 7 grams
		7½ hours	9.2	3.3	105	6.6	Calcium, 70 mgm
		Fasting	9.3	4.3	93	6.2	Phosphorus, 118 mgm.
		½ hour	9.5	3.5	104	6.1	
		2 hours	9.4	3.7	53	6.1	
		3½ hours	9.3	3.6	60	6.1	
		Lunch					
		7½ hours	9.0	3.6	200		

The results, in so far as the serum calcium and phosphorus levels are concerned, were essentially the same for ingestion of carbohydrate alone as when it followed the administration of insulin. In the latter cases, however, the blood sugar fell to reaction levels

In all subjects there was an early fall in serum phosphorus levels, averaging in eleven instances 0.8 mgm and varying from slightly less than 0.5 mgm to slightly more than 1.0 mgm. The initial height of the serum phosphorus seemed to have no influence on the magnitude of the change, nor did there seem to be any definite relation between the extent of the decrease in serum phosphorus and the change in the blood sugar level. When the phosphorus fell there was some tendency for the serum calcium to rise but this was by no means constant nor did the increase in serum calcium vary directly with the extent of decrease in serum phosphorus. With a maximum fall of about 1 mgm in serum phosphorus the serum calcium remained essentially constant in R F F, February 2, D M T, March 5, E R, March 5, and in DS (acromegaly). In the case B W (tetany), when the phosphorus fell from 7.5 to 6.5 the calcium remained essentially constant until the last determination, when, without further appreciable change in serum phosphorus it fell from 4.7 to 4.2. On the other hand, in R F F, June 26, the serum calcium rose from 9.6 to 10.4 mgm, while the phosphorus fell from 3.2 to 2.1. In eight hours, however, when the serum calcium had returned to 9.7 the serum phosphorus had risen to 4.2, 1 mgm higher than the fasting level. In D M T, June 27, when the serum phosphorus was decreased by 0.8 mgm the calcium rose from 9.2 to 9.6, reaching 9.8 one and one-half hours later when phosphorus had returned nearly to normal. In the case of hypertrichosis, the maximum rise in calcium was 0.9 mgm and maximum fall in phosphorus 0.4 mgm. The serum calcium was essentially unchanged in at least six of the eleven cases, and only in three cases was there a variation of 0.5 mgm or more, the greatest increase above fasting level being 1 mgm in R F F after carbohydrate and insulin. In the cases in which serum protein was determined there seemed to be no constant relation between the temporary variations in protein and those of calcium.

Thus, our results are in agreement with the well established fact that the ingestion of large amounts of carbohydrate with or without administration of insulin is constantly followed by a definite fall in serum phosphorus. They show on the whole little associated change in serum calcium, the magnitude of which was no greater than that found to occur on ordinary diet or after ingestion of other types of food.

as described below. Similar values for serum calcium in dogs, after administration of insulin, were obtained by Briggs, Koechig, Doisy and Weber (30). Brougher, however, reported tremendous changes in blood calcium of rabbits (rising even to more than 20 mgm) following injection of insulin (31). Davies, Dickens, and Dodds (32) found increases of from 2 to 4 mgm in serum calcium of rabbits in hypoglycemic convulsions. Recently, Ellsworth (33) has reported increases in serum calcium in man similar to those that occurred in some of our cases. The maximum change in calcium did not occur always in those cases with the greatest fall in phosphorus, nor was the reciprocal inverse relationship persistent in the last determinations of a given experiment. Yet in his cases there was a more constant rise in serum calcium than in ours.

#### *Ingestion of large amounts of protein and fat*

Data showing the effect of ingestion of large amounts of protein and fat are presented in table 6.

When 127 grams of fat were taken, much of it in the form of cream, there followed an increase of calcium from 9.6 to 10.8 mgm. and a fall in phosphorus from 3.9 to 3.2 mgm. A week later the effect of 100 grams of olive oil was observed, the serum phosphorus remained constant but serum calcium increased by 0.9 mgm in 4 hours. Similar experiments on ingestion of large amounts of protein, when the subject took 200 grams of protein daily for several days, showed in one instance a distinct rise in calcium from fasting value of 9.6 mgm to 11.4 mgm a few hours after breakfast, associated with a slight increase in serum phosphorus, but no material difference in serum protein. In this instance NaHCO<sub>3</sub> was added to neutralize the acid effect of ingested protein. When the experiment was subsequently repeated, leaving out the NaHCO<sub>3</sub>, the serum calcium remained stationary and serum phosphorus rose slightly. On each occasion the subject was carrying on with his ordinary work.

Data showing the effect of ingestion of single doses of phosphate and of calcium lactate are presented in table 7. The phosphate caused purgation, which persisted for several hours. The serum phosphorus, however, rose from 3.5 to 5.0 mgm, without an appreciable change in the serum calcium level. A similar rise in serum phosphorus after

ingestion of large amounts of phosphate without any associated change in the serum calcium level has been described by Schulz (34). The ingestion of calcium lactate was followed by an increase in serum

TABLE 6  
*Effect of ingestion of large amounts of protein and of fat*

Date	Time	Cal cium mgm per 100 cc	Phos phorus mgm per 100 cc	Pro tein per cent	Non protein nitrogen mgm per 100 cc	Remarks
1928	Fasting	9.6	3.9			Breakfast
	1 hour	10.5	3.2			Fat, 127 grams
	2½ hours	10.6	3.3			Carbohydrate, 15 grams
	5½ hours	10.8	3.6			Protein, 6 grams
	Lunch					Calcium, 185 mgm
	9 hours	10.0	3.7			Phosphorus, 181 mgm
February 20	Fasting	10.1	3.2			100 grams olive oil
	1 hour	10.8	3.2			
	2 hours	10.3	3.2			
	4 hours	11.0	3.1			
February 27	Fasting	9.6	3.6	6.3	31	Breakfast
	2½ hours	10.0	3.6	6.9	39	Protein, 61 grams
						Fat, 29 grams
						Calcium, 30 mgm
February 6						Phosphorus, 461 mgm.
	8 30 a m	9.6	3.2	6.4	45	Total diet for day
	9 45 a m	10.1	2.8	7.6	46	Protein, 200 grams
	10 00 a m	Breakfast, plus 2 grams soda				Carbohydrate, 161 grams
April 19	12 45 p m	11.4	3.7	6.9	51	Fat, 100 grams
	1 30 p m	Lunch, plus 3 grams soda				Calcium, 119 mgm
	2 30 p m	10.2	3.8	6.8	50	Phosphorus, 1,586 mgm
	6 30 p m	Dinner, plus 3 grams soda				
	9 15 p m	10.2	4.2		59	
April 26	8 50 a m	10.2	3.6	7.1	42	Same diet as on April 19
	9 00 a m	Breakfast				
	10 15 a m	10.0	3.7	6.8	50	
	12 15 p m	10.3	3.5	7.2	47	
	1 20 p m	10.2	3.3	6.8	50	
	1 30 p m	Lunch				
	6 15 p m	10.0	4.1	6.9	51	

calcium from 9.6 to 11.0 mgm but the inorganic phosphorus remained relatively constant

TABLE 7  
*Effect of ingestion of sodium phosphate and of calcium lactate*

Date	Time	Calcium mgm per 100 cc.	Phosphorus mgm per 100 cc.	Remarks
1928 June 19	Fasting	9.5	3.5	3 grams phosphorus in form of equimolecular solution of $\text{NaH}_2\text{PO}_4$ and $\text{Na}_2\text{HPO}_4$ . Purgation followed in about one hour
	1 hour	9.4	5.0	
	3½ hours	9.5	4.9	
	5 hours	9.2	5.0	
	Fasting	9.6	3.6	10 grams calcium lactate = 13 grams calcium
	2 hours	11.0	3.6	
	3 hours	10.5	3.6	
	5 hours	10.5	4.2*	
	Lunch			
	9 hours	10.3	3.8	

\* Some hemolysis.

#### COMMENT

It is seen that there may be greater changes in the serum calcium and phosphorus within a few hours after meals of ordinary foodstuffs than occur in the fasting levels over periods of many months. The fasting serum calcium, however, and to a lesser extent the serum phosphorus, remains remarkably constant in spite of great changes in diet and in the intake of calcium and phosphate.

Although the inverse relationship between the levels of calcium and phosphorus of the serum seems to hold in some instances during temporary fluctuations, it is quite clear that in many cases the calcium was constant while the phosphorus level changed, in others the phosphorus remained constant during changes in calcium, and in some instances both rose or fell together. Concomitant variation in the serum protein was not great enough to overshadow a specific effect of variation in phosphorus on the calcium level or vice versa, nor were temporary fluctuations in protein always paralleled by corresponding changes in serum calcium when the serum phosphorus remained constant.



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## GASTRIC SECRETION AFTER HISTAMINE SODIUM AND POTASSIUM CONTENT AND PEPSIN ESTIMATION

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Since Prout (1824) first demonstrated the presence of HCl in the gastric juice and Beaumont (1833) reported the demonstration by Dunglinson and Emmett of the bases, K, Na, Mg, and Ca, the composition of the secretion of the stomach has been repeatedly studied. The literature has been reviewed by Rosemann (1920), Carlson (1923), MacLean and Griffiths (1928), Gamble and McIver (1928), Bulger et al (1928) and McCann (1929). The recent introduction of histamine as a stimulant of gastric secretion following the work of Popielski (1920) and the subsequent studies, reviewed by Ivy, (1930) has made it possible to secure gastric juice undiluted by test meal. Pollard, Roberts and Bloomfield (1928) have demonstrated the value of measuring the rate of secretion of the constituents of the gastric juice following histamine stimulation. In their studies they measured the bases as total base. We have carried out similar studies fractionating the total base into its separate ions and report briefly our results.

From the studies in the literature it is clear that the course of gastric secretion whether stimulated by some type of test meal or by histamine is typically characterized by a rise in the curve of volume secretion associated normally in the mixed juice with rise in acid and chloride concentration, fall in sodium concentration (sodium constituting usually the larger part of the total base), approximate constancy of potassium concentration, concentrations of Ca and Mg always so small as to make difficult the measurement of their changes, fall of nitrogen concentration and change in character of the juice to a less mucoid, more watery consistency. Toward the close of the response to stimu-

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lation these changes are reversed. Fundic pouches give a similar picture but one that is free from the effects of swallowed saliva, mixture of secretion from various parts of the stomach, dilution and buffering by the test meal, and the occasional regurgitation of duodenal contents.

#### METHODS

In the gastro intestinal clinic of the Hospital of the University of Pennsylvania under the direction of Dr. F. Grier Miller patients were selected who had been thoroughly studied, usually after complete gastro-intestinal x-ray investigation. On the day of study the patient came without breakfast to the clinic. While reclining comfortably a small Rehfuss tube was cautiously introduced into the stomach and the fasting contents of the stomach removed as completely as possible by gravity and very gentle suction with a syringe. The subsequent fasting secretion collected continuously through the tube for 20 to 40 minutes was separated into samples representing 10 minute periods of collection. Either the specimens or the results of the analyses of the individual samples were combined to constitute "prehistamine" data. Histamine acid phosphate in a dose of 0.3 to 0.5 mgm as 1:1000 solution of the phosphate was injected subcutaneously. Continuous removal with partition of samples at 10 minute intervals was carried out until the response was subsiding, usually after 60 to 80 minutes. Volume of secretion and acid titration were measured on each specimen. The other analyses were made on certain specimens selected with respect to the volume and acid curve, the size of the specimen, and the freedom of the specimen from blood or bile. Chlorides were measured in all of the subjects, but K, Na, and N only in those indicated in the tables.

*Preparation.* Each specimen was measured in a cylinder, and passed through ash free filter paper before sampling for analysis.

*Acid titration* was performed on 1 cc samples with N/50 NaOH using Topfer's reagent to both the salmon and lemon end points, and phenolphthalein. The lemon end point of Topfer's was used in the human cases to determine the total HCl. This has been recommended by Michaelis (1926) and our data indicate in the human material that the sum of equivalents of total base and total HCl so measured approximately equals the equivalents of total chloride. It is evident that the further titration to the phenolphthalein end point represents for the most part combination of the NaOH with buffer substances in the specimen.

*Chlorides* were measured by Van Slyke's method as modified by Wilson and Ball (1928).

*Phosphates* were determined by an adaptation of the method of Fiske and Subbarow (1925) for total phosphorus. One cubic centimeter of juice was digested, with 2 cc of 2.5 N H<sub>2</sub>SO<sub>4</sub> and the later addition of 1 drop of concentrated HNO<sub>3</sub> in long Pyrex tubes, graduated at a volume of 10 cc. After partial dilution and

the addition of reagents, the solution was diluted to the 10 cc. mark. The unknown was compared in a colorimeter with a standard of approximately equal strength, three standards of different strengths being employed.

*Nitrogen* was determined by the Kjeldahl method.

*Bases* were determined after ashing a sample in silica or platinum beakers with  $H_2SO_4$  and superoxol the final stages being conducted in an electric oven at a temperature not exceeding 500°C. After complete disappearance of carbon, 1 drop of concentrated  $H_2SO_4$  acid was added to the ash and the heating in the oven repeated at the same temperature. The ash was dissolved in water made up to volume and aliquots taken for the following analyses.

*Total base* was measured by the method of Stadie and Ross (1925) on the ash from 1 cc. of juice.

*Potassium* was measured by the titrimetric method of Shohl and Bennett (1928) on the ash from 1 cc. of juice. Known solutions of potassium were analyzed in duplicate with each series.

*Sodium* was measured by the method of Barber and Kolthoff (1928) using Jena glass filtering crucibles for collection of the precipitates and employing the ash from 1 cc. of juice. Known solutions of sodium were analyzed in duplicate with each series.

*Pepsin* was estimated by a modification of the method of Pollard and Bloomfield (1929). In their method a number of tubes each containing 300 mgm. of edestin are prepared with increasing dilution of the unknown pepsin solution to constant total volume and acidity. They are permitted to digest in a water bath at 37°C for the same selected time measured to the minute. At the end of the period of digestion the remaining edestin is precipitated with trichloracetic acid, its volume read after centrifugation and the amount of edestin digested is estimated by difference from blank tubes containing edestin but no pepsin. To this point we have followed their method precisely as they described it. Pollard and Bloomfield then, in principle, divide the edestin digested by the amount of original pepsin solution present in the tube and plot this value, the edestin digested per unit of pepsin solution, against the dilution of the original pepsin solution in the tube. Such a plot rises to a maximum at some dilution and then, in their experience, falls. They take the edestin digested per unit of pepsin solution at the crest of this curve as a measure of the pepsin concentration of the unknown solution. We were unable to obtain reproducible estimates by this method of interpreting our data.

It is to be expected that, in the neighborhood of half digestion of the edestin, the relation of pepsin concentration to edestin digested will obey Schutz' law:

$$\log S = K + \frac{1}{2} \log E$$

where  $S$  is the amount of substrate digested,  $E$  is the concentration of enzyme, and  $K$  is a constant characteristic for the enzyme and substrate under constant conditions and for uniform time and temperature.

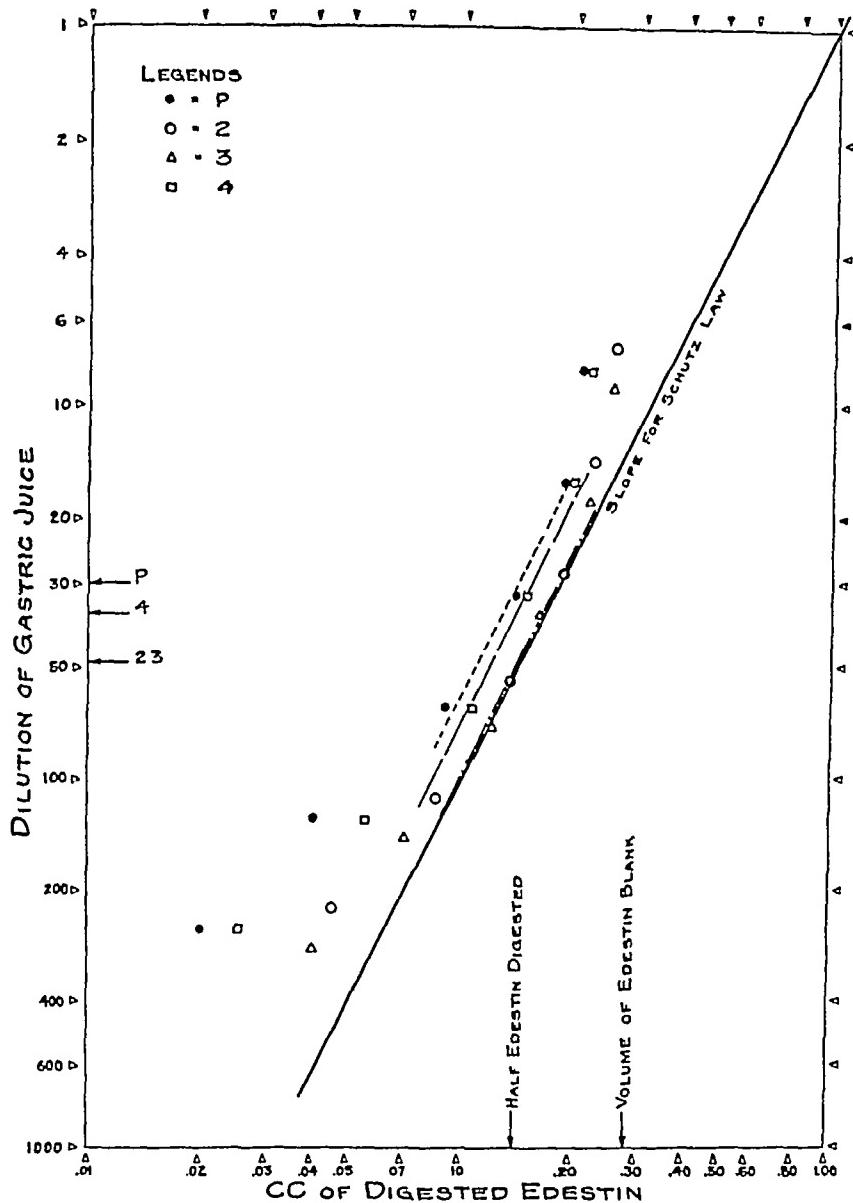


FIG 1 METHOD OF INTERPRETING PEPSIN READINGS (CASE BR, SPECIMENS P, 2, 3, AND 4)

For the range between 0.09 and 0.20 cc of edestin digested (abscissae) the values for each specimen approximate the slope of Schutz' law. The intersections of these slopes with the 0.14 ordinate representing half digestion of the edestin, give the dilutions shown by arrows on the left hand margin, which are taken as proportional to the concentration of pepsin in the original specimens.

TABLE I  
Clinical material

Case	Sex	Age	Weight	Nitro- gen mea- sured	Blood*	Bile	Diagnosis
High acid							
W	M	33	119	+			Cholelithiasis
AB	F	29	115	+			Visceroptosis
AE	M	37	137	+	Trace		Gastric ulcer
AL	M	44	115	+	Trace 3 to 7	+ in 1	Arteriosclerosis, hyper tension
AN	M	41	127	+	Traces	+3 and 5	Ulcers, gastroenterostomy, partial obstruction
AO	M	40	142	+		+4 to 6	Chronic constipation
AP	M	37	145	+			Diabetes mellitus, constipation
AQ	M	53	148				Hyperthyroidism, BM + 39 per cent
AZ	F	25	114	+			Chronic constipation
BF	F	41	152	+			Chronic cholecystitis
BI	F	47	130	+			Cholelithiasis
BK	M	33	147	+			Duodenal ulcer
BP	M	22	150	+		+2 to 4	Normal student
BQ	M	59	150	+		+P, trace later	Chronic constipation
BR	M	21	150	+	+3 to 6	+P	Normal student
BS	M	24	132	+	+2 to 5	+1, 4, 5	Normal student
BU	M	23	140	+			Normal student
Low acid							
B	M	61	111		Traces		Tuberculosis (?), anemia
U	M	53	158	+			Arteriosclerosis
V	M	23	150	+			Duodenal ulcer
AR	M	71	116				Carcinoma stomach, resection
AT	F	48	155	+	Traces		Cholelithiasis
AU	M	43	127		++ except 4		Carcinoma stomach
AV	M	62	190	+			Chronic constipation
BD	F	25	115	+			Duodenal adhesions
BE	M	48	147				Chronic arthritis
BJ	F	44	146				Intestinal adhesions
BO	M	22	150				Normal student

\* Figures refer to the fractional 10 minute periods following histamine in which blood or bile appeared, P indicating the prehistamine period

TABLE 2  
Summary of analyses on clinical material

Period.	High acid group Maximum total acid > 60, 18 cases								Low acid group Maximum total acid < 60 13 cases								
	P	1	2	3	4	5	6	7	P	1	2	3	4	5	6	7	8
Amount, cc per 10 minutes																	
Maximum	29	24	37	59	36	58	21	27	30	50	44	29	23	16	12	22	
Minimum	5	2	5	8	5	2	4	2	1	1	1	1	1	4	3	2	
Average	12	14	23	25	21	13	—	—	12	19	17	15	10	—	9	—	
Cl, m Eq per liter																	
Maximum	131	148	159	156	156	147	—	—	127	132	132	134	133	—	136	—	
Minimum	67	99	64	107	95	75	—	—	68	68	57	57	68	—	58	—	
Average	105	123	133	137	138	126	—	—	89	98	96	95	95	—	94	—	
Total acid m Eq per liter																	
Maximum	81	—	—	116*	—	—	—	—	35	47*	—	—	—	—	—	—	—
Minimum	—	13	64*	—	99*	—	—	—	—25	—4	—	—	—	—	—	—	—
Average	30	15	15	—	—	13	—	—	—	—	—	—	—	—	—	—	—
K, m Eq per liter, average																	
Na, m Eq per 10 minutes, average	0.45	0.49	0.49	0.57	0.57	0.55	0.55	0.58	0.29	0.36	0.49	0.43	0.43	0.49	0.51	0.51	0.51
N, m Eq (NH <sub>3</sub> ) per 10 minutes, average	0	0	0	0	0	0	0	0	0.81	0	0	0	0	0	0	0	0

\*Crest of acid curve

If the edestin digested be plotted on logarithmic paper against the dilution of unknown solution, then in the neighborhood of half digestion of the edestin the data should fall along a straight line whose slope is given by the equation

TABLE 3  
Potassium concentration (*m Eq per liter*)

Case	P	1	2	3	4	5	6	7	8
	Prehistamine	0-10 minutes	10-20 minutes	20-30 minutes	30-40 minutes	40-50 minutes	50-60 minutes	60-70 minutes	70-80 minutes

High acid

W	10	8	10			10			
AE	15	14		14			16*		
AL	14		13			7			
AN	14		17	15			15		
AO	10	14	18			15			
AP	17	19	32			13		14	
AQ								14	
AZ			13				14		
BF							13		
BP	22	21	23	20					
BQ				13					
BR	17		18	17	16				
BS	14	11	17		15				
BU	17			16					

Low acid

U				11					
V	9	9		10			5		
AR							5		
AT	16	15		16			15	14	
AU	15	13		16					
AV	13								
BD									
BE							17		
BO	11		17	16			16		
								18	

\* Broken columns in this and other tables indicate a combination of material from several periods, 6, 7 and 8 in this instance

Figure 1 shows that in the neighborhood of half digestion of the edestin the observations exhibit this relationship. A line whose slope is that of the equation is accordingly drawn through the points near the locus of half digestion. The dilution indicated by this line at half digestion is taken as the concentration of pepsin in arbitrary units in the original unknown solution.

## RESULTS OF CLINICAL STUDIES

The patients studied are listed in table 1 with respect to sex, age, and diagnosis. All were used in studying volume rate of secretion, acid titration and chlorides, but only those indicated in tables 1, 3, and 4 for the measurement of N, K, and Na. In no instances were these latter measurements made on specimens containing more than traces

TABLE 4  
*Sodium excretion (m Eq per liter)*

Case	P	1	2	3	4	5	6	7	8
	Prehistamine	0-10 minutes	10-20 minutes	20-30 minutes	30-40 minutes	40-50 minutes	50-60 minutes	60-70 minutes	70-80 minutes
High acid									
W	65	62	14			20			
AB	64	70	35		27	20			
AE	29	38		14				16	
AL	80		39			61			
AN	31		23						40
AO	42	38	22			11			
AP	42	17	8			3		6	
BP	19	14	4	2					
BQ			56		13	13			
BR	50		23	12	8				
BU	20				36				
Low acid									
B	49			31					
U	56	56		29		30			
V	73	65		54		46			
BO	27		32	24					37

of bile or blood and usually the specimens employed were not visibly contaminated with either

Examination of our data revealed no significant correlation with the diagnoses accepted other than the well recognized correlation of certain conditions with high or low acid titrations. Our material has been divided arbitrarily into those subjects whose maximum total HCl acid titration (lemon end point with Topfer's reagent) exceeded 60 m Eq per liter and those failing to reach this acidity. In dealing

with each component we direct attention either to its concentration or to its rate of secretion depending upon which proves to be as a rule the more constant through the period of response to stimulus. Following this criterion we have directed attention to the concentration of chloride and of potassium and to the rate of secretion of sodium, nitrogen, and acid. The results with respect to these components are shown in summary in table 2, supplemented by tables 3 and 4 and

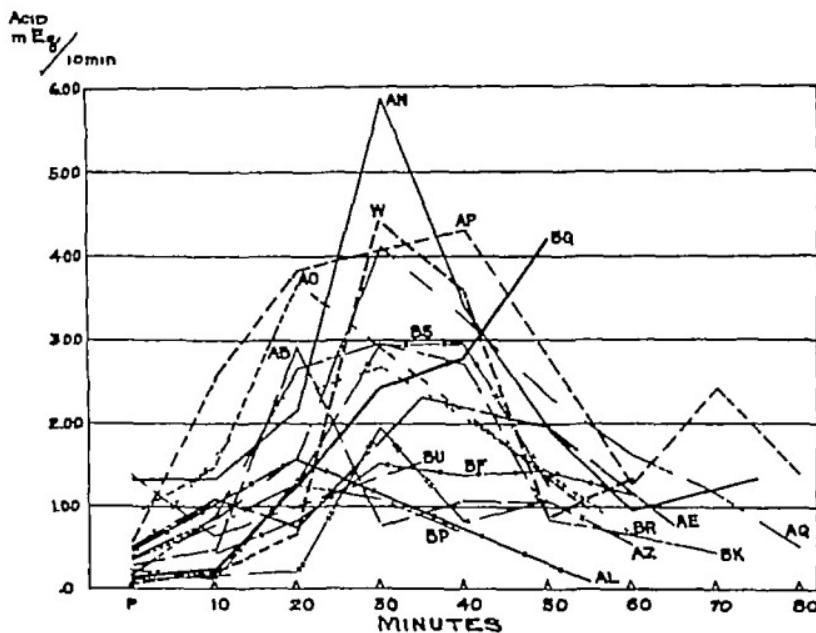


FIG. 2 RATE OF ACID SECRETION IN HIGH ACID GROUP

figures 2 to 7, for it is clear that the significance of the course of the individual curves is somewhat masked in the presentation of table 2.

In the high acid group, the maximum response is about 30 minutes after histamine, with rise of  $\text{Cl}^-$  and acid concentrations and fall of  $\text{Na}^+$  and N concentrations. The rate of  $\text{Na}^+$  and N secretion are usually slightly increased but the crest of these curves is earlier, usually 10 to 20 minutes after the stimulus.

In the low acid group the volume secretion rises during the first 10

minutes, as in the high acid group, but then begins to fall off. The difference between the two groups lies not only in the marked difference

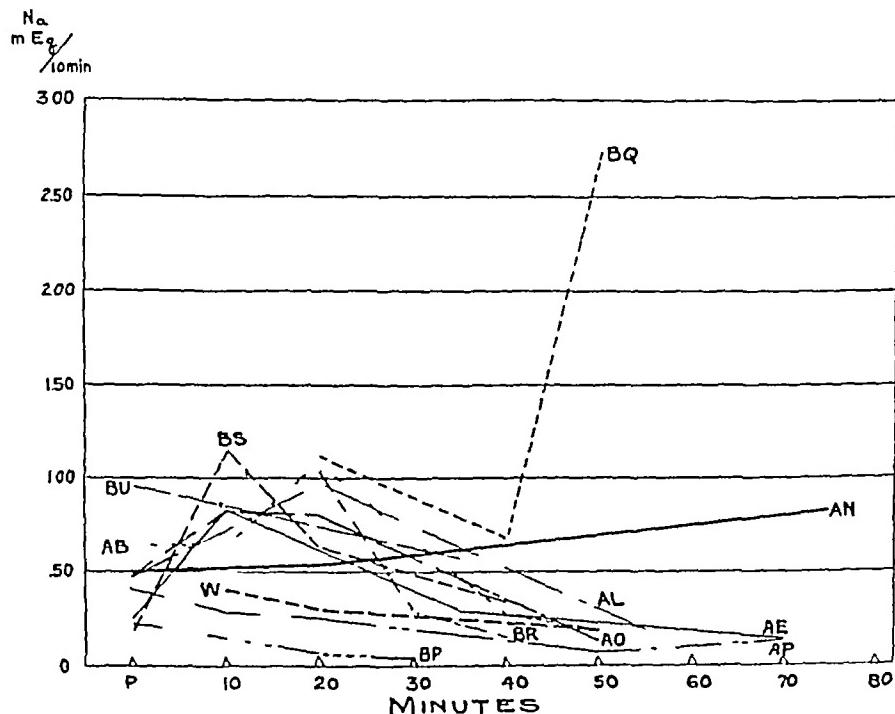


FIG. 3 RATE OF SODIUM SECRETION IN HIGH ACID GROUP

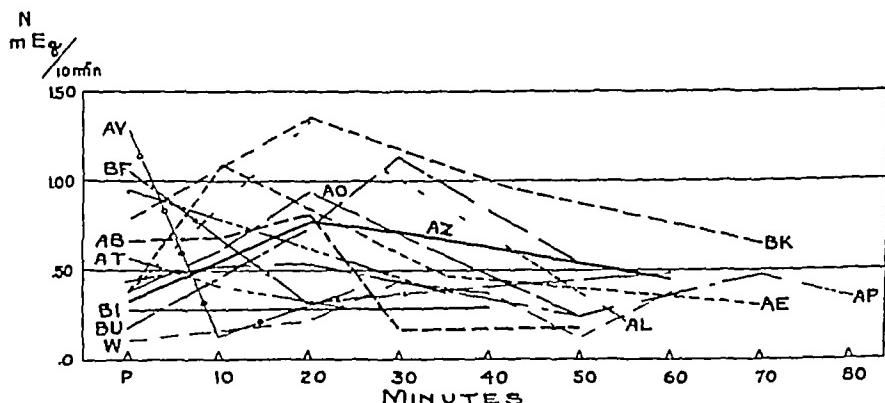


FIG. 4 RATE OF NITROGEN SECRETION EXPRESSED AS  $m\text{ Eq. } \text{NH}_3$  IN HIGH ACID GROUP

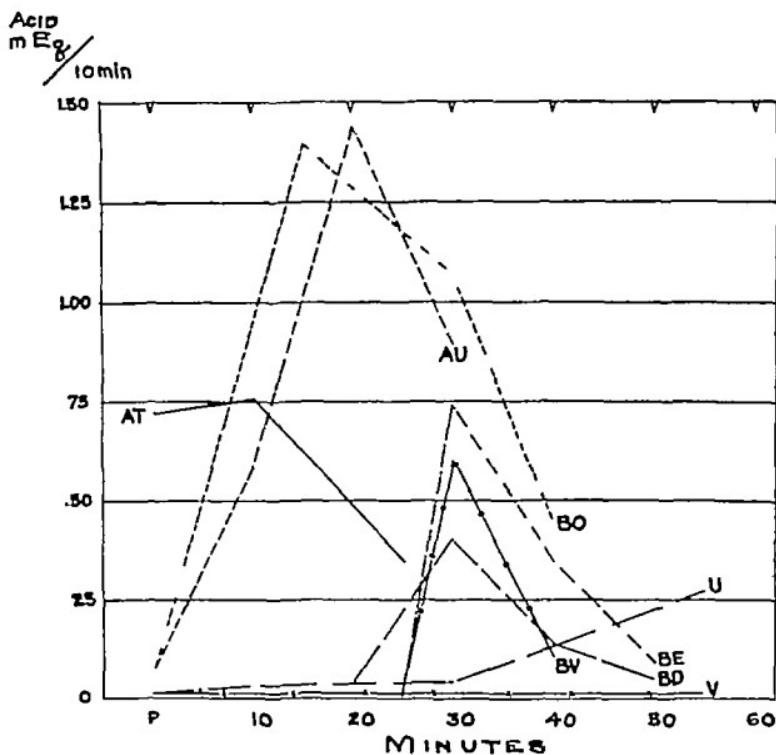


FIG. 5 RATE OF ACID SECRETION IN LOW ACID GROUP  
Note that ordinates are on a larger scale than those of figure 2

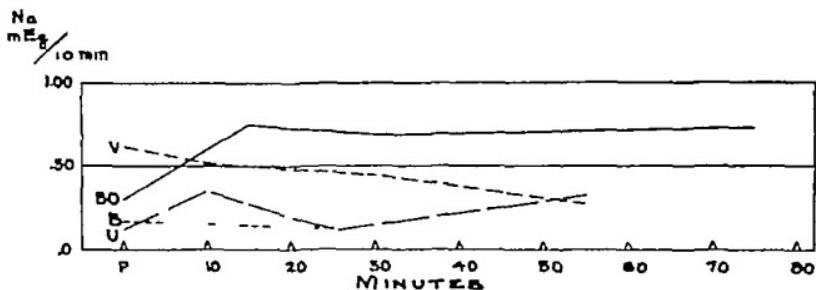


FIG. 6 RATE OF SODIUM SECRETION IN LOW ACID GROUP

in rate of HCl secretion but in less tendency to a rise in rate of secretion of Na and N in the low acid group. The correlation between N and  $\text{Na}^+$  secretion is a rather striking one as shown in figure 8. Since in this graph rates of secretion and not concentrations are compared a misleading apparent correlation due to dilution of both by increased secretion of HCl is avoided.

The  $\text{K}^+$  concentration is relatively constant throughout. The chloride concentration approaches at its maximum the figures of Carl-

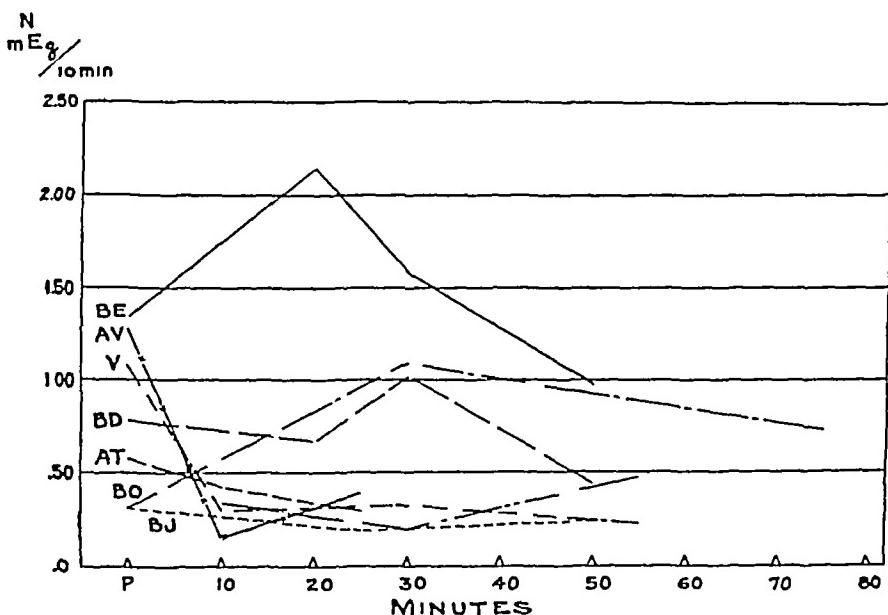


FIG 7 RATE OF NITROGEN SECRETION EXPRESSED AS m Eq.  $\text{NH}_3$  IN LOW ACID GROUP

son (1923) (150–165 m Eq per liter) on gastric fistula juice from man and of Gamble and McIver (1928) on juice from fundic pouches in dogs but never quite reaches these values. In the periods of lower stimulation, and especially in the low acid group, it may reach only 35 per cent of these values. In the low acid group, a chloride concentration as low as 64 m Eq per liter was observed in a subject with active secretion amounting to 31 cc per 10 minutes. If we interpret the gastric secretion as consisting of a mixture of an acid secretion

containing HCl 135 m Eq per liter and KCl 15 m Eq per liter and a second mucoid secretion containing mucus, nitrogen, and NaCl, the evidence suggests that the NaCl concentrations in the mucoid secretion averages about 50 m Eq per liter, which is much lower than the HCl concentration of the acid secretion, the low acid group under this interpretation is characterized by marked depression of the HCl secre-

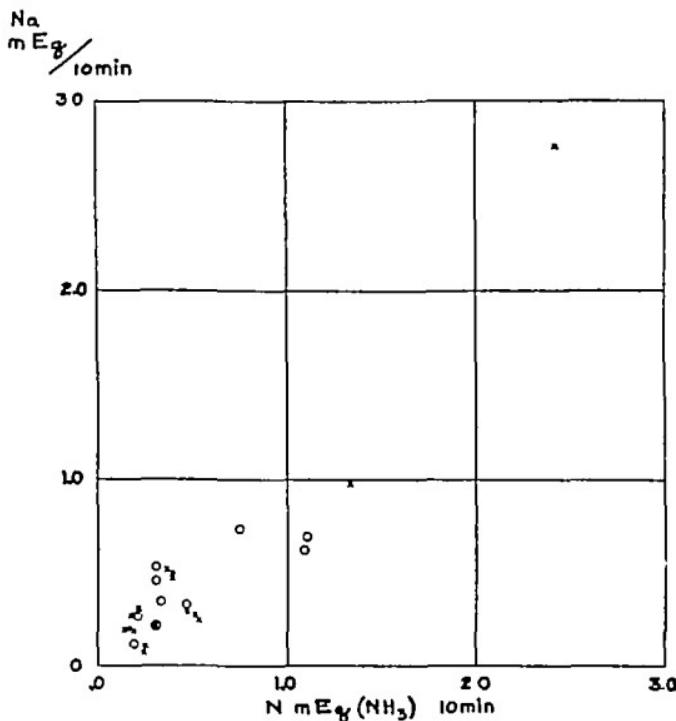


TABLE 5  
*Secretion from fundus, pyloric end, and duodenum*

	Time	Amount	Rate	Acid titration		Cl	K	Na	N	Acid secretion	Na secretion
				Topfer	Phenolphthalein						
Fundus	10 59-11 23	0	0	m Eq per liter	m Eq per liter	m Eq per liter	m Eq per liter	m Eq per liter	m Eq per liter	m Eq per liter	m Eq per liter
	11 23-11 34	10	9 1	38	60	72	151	14	34	42	0 54
	11 34-11 44	11	11 0	93	110	118	157	14	34	20	1 21
	11 44-11 59	16	10 7	121	134	139					0 37
	11 59-12 11	11	9 2	129	141	146					1 43
	12 11-12 22	13	11 8	125	141	146	158	11	17	8	1 30
Pylorus*	10 59-12 22	3	0 36	-23	-12	2	141	29	156	60	0 56
Duodenum	10 59-12 22	14	1 69	-87	-46	2	117	14	163	41	0 28

\* Because the specimens for analysis were small and measured with great difficulty owing to the highly viscous mucus, these figures are only roughly approximate.

ually. The difference, which includes the sum of the errors of the three methods, ranges from -4 to +15 m.Eq per liter with a mean of +4.04. This mean figure agrees approximately with the few analyses made for Ca, giving about 2 m.Eq per liter, and for Mg, giving about 1 m.Eq per liter. Total base concentrations in our data range from 18 to 101 m.Eq per liter with variations from period to period in a given fractional study of from 10 to 65 m.Eq per liter. Attention directed to total base concentration seems to us less illuminating than to K<sup>+</sup> concentration and rate of Na<sup>+</sup> secretion. Total phosphorus was measured in 18 specimens from 6 of the early cases studied. In 11 specimens from 3.0 to 0.2 mM per liter of PO<sub>4</sub> was found, and in 7, less than 0.1 mM per liter. It was higher in the fasting contents. It was not followed further.

We have measured the pepsin concentration in several of our observations following histamine stimulation. It suffices to record that no consistent correlation of pepsin concentration with curve of secretion or acidity was observed. The pepsin concentration was as a rule more constant than either rate of secretion or acidity. Sodium concentration varied from 2 to 81 m.Eq per liter.

That histamine stimulates frequently the secretion of a juice high in HCl, undiluted by test meal, is apparent both in our studies and in those of others. As will be pointed out by Gammon and Miller (1931) in a more extensive clinical study of the histamine test it is probable that the conditions of the histamine test, lacking as it does any diluting meal, do not afford opportunity for demonstrating the tendency, which Michaelis (1926) points out as characteristic of the normal stomach, of secreting enough acid to bring the pH of its contents to a relatively constant value, indicated by a free HCl of about 20 to 40 m.Eq per liter. If this be true, a most important regulating mechanism, highly characteristic of normal gastric function, is masked under the conditions of the histamine test.

#### EXPERIMENTAL STUDIES

For comparison with the clinical material, we studied with the same chemical methods the secretion of fundic pouches in dogs after stimulation by food and by histamine. For the preparation of these pouches we are indebted to Dr. I. S. Ravdin, Professor of Surgical Research.

Dog I, male, weight 13 kilos, was operated upon on October 4, 1929 by Dr I S Ravdin who constructed a Pawlow pouch in the fundus of the stomach. Juice was collected during the periods of study by continuous aspiration through a fenestrated tube introduced into the pouch and leading to a collecting bottle. The animal was fed once daily on an adequate ration of casein, sugar, lard, salts, and vitavose. Weight was maintained approximately constant and the dog remained in good condition.

Three feeding experiments (IF1, IF2, and IF3) were conducted, in each of which, after commencing the collection of pouch contents, the daily feeding was given and the contents of the pouch collected, for 24 hours in the first two experiments and 10½ hours in the third. These experiments were conducted on November 15, November 25, 1929 and January 2, 1930. Two histamine experiments (IH1, and IH2) were conducted on November 19, and November 22, 1929. In these experiments the collection from the pouch was commenced in the fasting dog, histamine acid phosphate was injected subcutaneously. On November 19th, 5 cc of 1:1000 solution were injected which resulted in marked conjunctival congestion and excitement, on November 22nd, 3 cc of 1:1000 solution were injected and resulted in slight conjunctival congestion and a mild degree of restlessness. The dog drank water frequently. The collection was continued until the secretion had ceased which occurred after 3½ and 2½ hours respectively.

Dog II, male, weight 11.5 kilos, was operated upon on October 11, 1929 by Dr I S Ravdin and a Heidenhain pouch constructed in the fundus of the stomach. Studies were conducted in a manner similar to those on Dog I. Two feeding experiments (IIF1 and IIF2) were performed on November 15, and 25, 1929, and a histamine experiment (IIH1) on November 22, 1929. In the latter, 3 cc of 1:1000 solution of histamine were given subcutaneously and resulted in conjunctival congestion, mild excitement, slight incoordination, and apparent confusion.

Because of great variation in rate of secretion it was not considered practical to divide the collection over predetermined intervals. Instead the periods of collection were closed as sufficient juice for analysis had accumulated, the collecting tubes being emptied as completely as possible at the close of each period and the time of each period noted. The tabulation of the results is rendered somewhat difficult for this reason. We have accordingly prepared Chart 1 in which each of the components is presented for each period of each experiment either with respect to concentration or rate of secretion in horizontal blocks which represent the duration of the period and its location in time with respect to the stimulation by food or histamine. In only one experiment, IF3, were we able to collect any secretion before stimulation.

	P	Z	4	6	HOURS	10	12	24
Ma.	IF1	0	45	160	109		118	77
E	IF2							
	IF3	360	360	357	300	264		
	IF1	0	307	280	508	243	169	141
	IF2							
DAY	TH1	0	NA	109				
	TH2	0	416					
Kilo.	TH1	H 600	—	—	260			
N	IF1	0	72	152	—	118		54
E	IF2	2120	158	110	116	79		
	IF3	800	260	350	330	240		
	IF1	0	214	—	109	—	104	
	IF2	0	245	228	224	—		
DAY	TH1	0	NA	87				
	TH2	0	355					
Kilo.	TH1	H 600	—	—	250			
No.	IF1	—	14	16	—	0	0	
E	IF2			6				
	IF3	0	23	20	—	17	67	
	IF1	—	—	—				
L	TH1	—	8					
	TH2	—	10					
	TH1	—	—	—				
N	IF1	—	16	—	5	—	24	
mE	IF2	—	17	6	6	—	8	
	IF3	86	27	28	—			
	IF1	—	18	—	14	—	75	
	IF2	—	30	—	35	—		
L	TH1	—	18					
	TH2	—	8					
	TH1	—	11	—	—	43		
TOTAL	IF1	0	0.08	0.0	1.08	2.05	1.44	0.48
Ac	IF2	0.023	24	200	2.10	140	0.06	
mE	IF3	0.03	155	148	1.08	0.95	0.05	
	IF1	0	1.54	1.89	1.06	1.78	0.97	0.15
	IF2	0.078	0.88	—	0.74	0.08		
DAY	TH1	0	NA	1.01				
	TH2	0	0.77	0.14				
Kilo.	TH1	0	103	108	0.04	—	0.02	
	TH2	0	103	108	—			
Am	TH1	—	NA	1.02				
	TH2	—	103	100				
L	TH1	—	11	145	—	80		
	TH2	—	11	145	—	80		
AMT	IF1	0	4.5	8.0	11.4	4.7	6.0	3.0
CC	IF2	0.17	1.5	1.8	1.5	4.5	4.8	0.8
	IF3	0.5	1.31	1.31	1.23	NA	0.8	1.8
DAY	IF1	0	1.5	4.0	15.3	18.8	7.6	2.1
	IF2	0.03	NA	7.5	6.4	—	0.8	
	TH1	0.04	125					
Kilo.	TH2	0	1.42	0.0				
	TH1	0	1.73	1.73	0.8	—	5.0	
CL	IF1	—	155	155	155	155	155	155
mE	IF2	—	163	167	167	166	166	166
	IF3	152	157	184	136	—		
	IF1	—	163	155	159	155	155	146
	IF2	—	163	—	166	—		
L	TH1	—	NA	161				
	TH2	—	166					
	TH1	—	170	165	—	154		
K	IF1	—	14	16	—	0	0	
mE	IF2							
	IF3	0	13	12	—	7	7	
	IF1	—	—	—				
L	TH1	0	5					
	TH2	0	7	—	—	0		
	TH1	0	7	—	—	0		

CHART 1 EXPERIMENTS ON FUNDIC POUCHES IN DOG I, PAWLOW POUCH, AND DOG II, HEIDENHAIN POUCH

F indicates stimulation by feeding, H, stimulation by histamine P indicates the time of stimulation. Figures show rate of excretion or concentration of the various constituents for the period in hours indicated by the position and length of the block in which the figure is placed. Zero time, "P," indicates the time of feeding or giving histamine. Dash in a block indicates no measurement made during that period.

## RESULTS

*Feeding experiments*

*Rate of secretion* The secretion, except in experiment IIF3, began from 10 to 90 minutes after feeding. In each animal the secretion began earlier in successive experiments, which suggests that nervous factors may have delayed the beginning of secretion in the earlier experiments. The secretion when established was of many hours duration and was usually maximal at about 4 to 10 hours after feeding.

*Chloride concentration* remained almost constant through most of each experiment but there was a moderate fall toward the end of two of the experiments, IIF3 and IIF1.

*Total HCl* was measured to the phenolphthalein end point. Differing in this respect from our studies on human gastric juice, in the juice from the pouches the sum of the equivalents of total HCl measured to phenolphthalein plus equivalents of total base agreed more closely with the total chloride than did the sum of base and the total HCl measured to the lemon point of Topfer's indicator. Buffer substances in the juice from the pouches and in our human gastric juice would seem therefore to differ. Total HCl ranged in concentration from 72 to 145 m Eq per liter and was always above 91 in the first juice collected. Free HCl values were from 47 to 136 and above 68 in the first juice collected. As could be expected from the moderate variation in acid concentration the rate of acid secretion followed approximately the rate of volume secretion.

*Potassium concentration* when studied ranged from 6 to 16 m Eq per liter with a tendency to lower values toward the close of the period of secretion.

*Sodium secretion* followed almost the same curve as the acid and volume secretion, with a disproportionately high secretion in the resting 12 hour period studied at the end of IIF1. In this period the Na concentration was 67 m Eq per liter. Otherwise the concentrations were from 6 to 25 m Eq per liter.

*Nitrogen secretion* was closely correlated with the Na secretion and both were high when the juice was conspicuously mucoid in character.

*Histamine experiments*

These experiments were characterized by an extraordinarily great outpouring of secretion during the first hour at a rate three or four times the maximum attained after feeding but ceasing after 2½ to 3½ hours. The composition of the secretion was not significantly different from that which followed feeding. The earlier crest of sodium than of acid secretion which we noted in the high acid group of our clinical material is manifested by the data on Na concentration in experiment IH1 and by the fact that the maximal acid concentration occurred always in the period following the maximum rate of volume secretion and of sodium concentration and secretion.

*Experiment designed to separate secretion from fundus, pyloric end of stomach and duodenum*

*Protocol* On April 23, 1930, a dog of about 12 kilos weight which had been fasted 12 hours was anesthetized with amytal anesthesia. At 10 A.M. the abdomen was opened. Four long jawed clamps protected with rubber were applied to divide the lumen of stomach and duodenum into three portions, care being taken to interfere as little as possible with the blood supply. The first closed the oesophagus at its entrance to the stomach. The second divided the fundus from the pyloric end of the stomach along a line from the angulation of the lesser curvature to the junction of the splenic and pancreatico-duodenal arterial supply of the greater curvature. The third occluded the duodenum at the junction of first and second parts. The fourth was placed a little below the ampulla of Vater. The common bile duct was ligated. Bile was excluded from the duodenum, but not pancreatic juice. Three small incisions were made admitting fenestrated tubes into the fundus, the pyloric end of stomach, and the duodenum opposite the ampulla of Vater respectively. At 10 55 the stomach was emptied. At 10 59 histamine acid phosphate, 2.5 cc. of 1:1000 solution, was injected subcutaneously. Continuous aspiration from the three tubes was maintained until 12 22 with collection of secretion as shown in table 5. Violent peristalsis was noted in stomach and duodenum beginning about 11 02 and persisting with varying intensity for most of the experiment.

*Results* In table 5 are collected the analytical data. It is evident in this experiment that the secretion from the pyloric end was negligible in quantity consisting of thick mucus. The secretion of the fundus was similar in character to that of our clinical experiments and fundus pouches. It exhibited the crest of Na secretion early, followed by maximal acid secretion later, as in our other experiments.

It also showed the correlation of  $\text{Na}^+$  and N and the more constant  $\text{K}^+$  concentration

#### SUMMARY

The studies presented furnish direct measurement of the rate of Na and K secretion following histamine stimulation in patients and after stimulation by both feeding and histamine in fundic pouches in dogs

They indicate that the period of maximal sodium secretion after histamine is before the period of maximal acid secretion and not as the acid secretion is subsiding. A late increase in Na secretion was inferred by MacLean and Griffiths (1928) from the increase in neutral chloride in the gastric juice during subsidence of acid secretion following various types of stimulus other than histamine. The occasional increase in sodium concentration in this period in our studies is due to fall in acid secretion and not due to increased rate of sodium secretion.

The rate of nitrogen and sodium secretion in the low acid group is approximately the same as in the high acid group, not any higher.

Potassium from its constancy of concentration appears to be a constituent in about the same concentration in the gastric secretion whether this be highly acid or neutral.

The correlation in rate of secretion of sodium and of nitrogen suggests that they are constituents of one part of the gastric secretion, a part which appears to be mucoid in character.

Our results appear to be consistent with the studies of Gamble and McIver (1928) and Pollard, Roberts, and Bloomfield (1928).

The course of secretion in the fundus pouches is sufficiently like that in the gastric contents of our patients to suggest that the major factor in determining the character of the gastric secretion obtained in our patients is the secretion of the fundus.

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## STUDIES ON THE NITROGEN AND SULPHUR METABOLISM IN "BRIGHT'S DISEASE"

### II. OBSERVATIONS ON THE NITROGEN AND SULPHUR EXCRETION IN PATIENTS WITHOUT RENAL EDEMA<sup>1</sup>

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In the course of the studies on the nitrogen and sulphur excretion in Bright's Disease, we early found one patient who seemed to be constantly in negative sulphur balance. While this patient was a moderately advanced nephritic at the time of study, he differed in no essential particular from other patients with Bright's Disease and nitrogen retention. A section of the experiment on this patient is shown in figure 1. During this entire period this patient was in positive nitrogen balance, was on a satisfactory diet in every possible respect, and was eating over two thousand calories. This led us to search the other records for evidence of a similar condition, and we have been able to find in every patient with chronic nephritis without renal edema, periods of actual negative sulphur balance, taking into account the urine alone. In many experiments on normals, the protocols of some of which are available in another publication (1), no such negative balance even for one day was ever encountered. In a patient studied later, we happened to find a period of transition from positive to negative sulphur balance. Nothing in the clinical condition of the patient could be found to account for this change. A portion of the experiment is shown in figure 2.

The plan of experiment was similar to that used in the previous study on patients with "nephrosis" (2). All these patients were on weighed diets. The food intake was not analyzed, but standard

<sup>1</sup> This work was done under a grant from the Proctor Fund for the Study of Chronic Disease of Harvard University

tables of composition were employed to calculate the dietary constituents including the water in the food. To prevent a possible diuretic effect of water the total water intake, including the water in the food, was kept constant. Refusals of food were weighed on the ward and the total intake calculated by subtraction. The intake was essentially

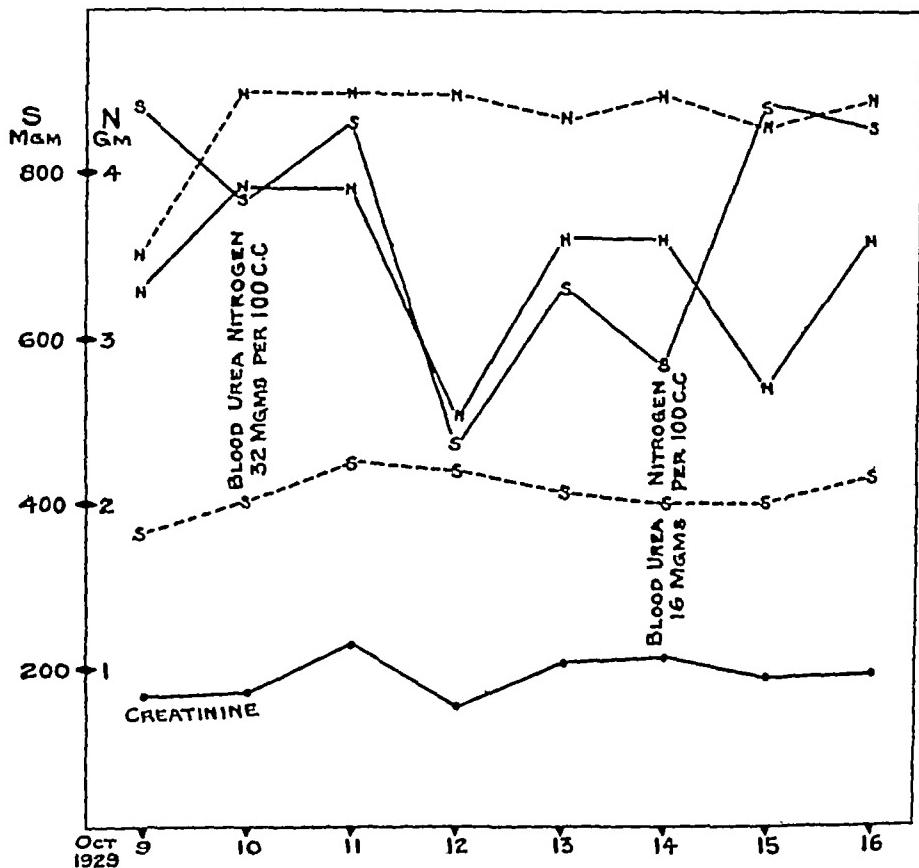


FIG 1 CASE X SHOWS CONSTANTLY NEGATIVE SULPHUR BALANCE  
Solid lines show urinary output, dotted lines show intake

constant as to nitrogen, sulphur, phosphorus, calories and water. The diets were varied according to therapeutic necessity in the various patients, and corresponded to the standard hospital diets containing 28, 40 and 60 grams of protein. We thus had the advantage of keeping the patient on an approximate diet for a long time before the more

laborious process of carefully weighing the foods was commenced. In no period presented, was the patient on a weighed diet less than ten days. All diets contained about 2000 calories made up largely by carbohydrate, as the fat intake never rose above 80 grams. The

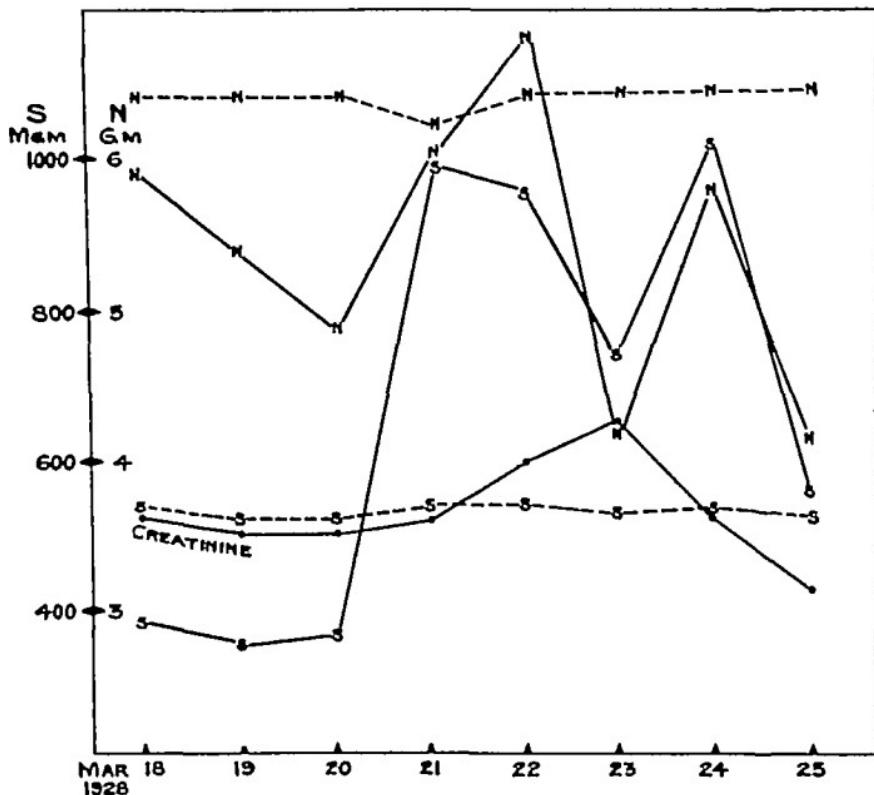


FIG 2 CASE VI SHOWS TRANSITION FROM POSITIVE TO NEGATIVE SULPHUR BALANCE

Solid lines show urinary output, dotted lines show intake

nitrogen, sulphur and phosphorus ran almost exactly parallel and the levels are sufficiently indicated in the figures.

Analyses of the urine were done daily for creatinine by the Folin method (3), total nitrogen by the Folin modification of the Kjeldahl method (3) and total sulphur by Fiske's benzidine method (4). For

technical reasons the sulphur and nitrogen of the feces was not determined. The addition of this figure would merely emphasize the negative sulphur balance and would increase the number of days in

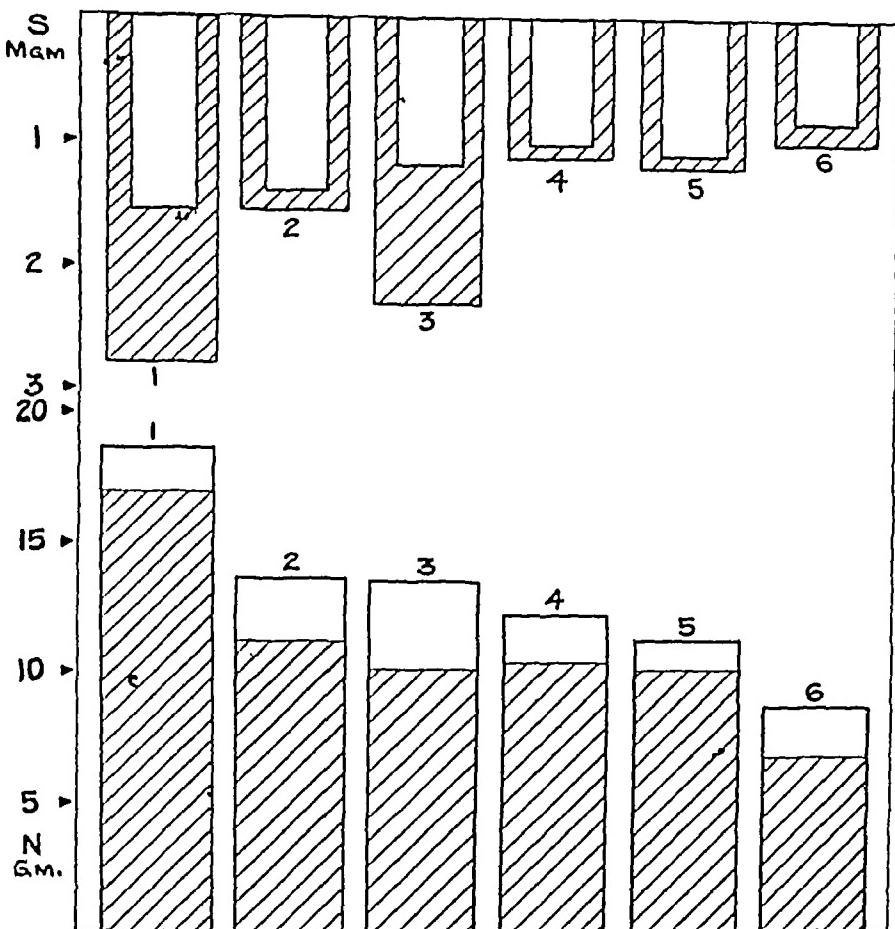


FIG 3 NITROGEN (LOWER) AND SULPHUR (UPPER) INTAKE AND OUTPUT IN SIX PATIENTS WITH CHRONIC NEPHRITIS WITHOUT RENAL EDEMA

Each column represents three days. Shaded sections indicate output, unshaded intake. Number of period (cf table 1) shown at each column

which actual negative balance existed in the bodies of these patients, as there were a good many where the sulphur excretion in the urine alone was almost equal to the intake

In figure 3 are shown the nitrogen and sulphur output and intake in selected periods from six patients with chronic nephritis, without renal edema. The shaded portion of the figure represents the output, the unshaded portion the intake. Each column represents a three day period. The dates of the periods of study are indicated in the table and correlation with the patient's clinical condition may be made by reference to the appended case histories. These are arranged, as were those previously presented (2), in a descending series according

TABLE I

*Additional data on periods shown in figure 3 and data on patients while in positive sulphur balance*

Period on chart	Case number	Dates of period	$\frac{N}{S}$ intake	$\frac{N}{S}$ output	Blood urea nitrogen	Phenol sulphon phthalein test	Blood pressure	
							Systolic	Diastolic
1	VI	March 22-25	11.8	6.0	mgm. per 100 cc	per cent	mm. Hg	mm. Hg
2	XI	October 26-29	9.6	7.2	10	60	120	82
3	X	October 14-17	10.8	4.3	16	10	160-182	90-110
4	VII	April 7-10	11.9	9.1	7	22	160	114
5	IX	August 18-21	10.1	8.7	13	20	224	142
6	VIII	January 14-17	10.4	6.9	18	10	120	80
*	VI	March 13-14	12.8	13.8		42	120	80
	VI	March 20-21	12.6	13.1		40	120	80
	VII	March 2-3	12.6	10	15	14	223	162
	VII	April 4-5	13.6	11.5	7		160	114
	VII	March 31-April 1	12.5	13.4		32	150	108
	VIII	January 25-26	11.1	22.3	13	25	104	68
	IX	August 27-28	14.6	9.2	15	20	104	68

\* These periods are not charted but indicate the  $\frac{N}{S}$  ratio during positive sulphur balance.

to the nitrogen intake. There it will be noted that the relationship of nitrogen in the urine to the intake is approximately that usually stated to be normal, whereas the sulphur output exceeded the intake distinctly and in two cases by a very considerable amount. It is perhaps worthwhile to call attention to the nitrogen sulphur ratios in this experiment. It is obvious that they are very low indeed. We have also compared the ratios during this period of negative sulphur balance with the nitrogen sulphur ratios during periods of more nor-

mal sulphur excretion (cf table 1) We found that the tendency was, on the whole, for the nitrogen-sulphur ratios to be low or normal and in only one case were we able to find a high ratio comparable to those reported in patients with "nephrosis" The nitrogen-sulphur ratio of the intake, of course, was approximately the same It is obvious that we have a striking contrast here, to the picture shown by patients with the nephrosis syndrome

In the previous work, it was pointed out that the type of protein excreted during the nephrosis syndrome suggests a mobilization and excretion of deposit protein Here the reverse appears to be the case It seems unlikely that we are dealing only with a variation in the selective secretion of nitrogen and sulphur dependent solely on the kidney lesion For example, in the first case charted, if the excretion of sulphur be taken to represent protein, we must account for the nitrogen left behind when this extra sulphur is excreted Were this to be represented in the form of nonprotein nitrogen, the nonprotein nitrogen of this patient's blood should have risen on the average of 4 mgm per 100 cc per day during the period pictured on the chart This did not occur Of course, such nitrogen may be in the tissues or a type of deposit nitrogen may have been built up poor in sulphur All this, however, is entirely hypothetical but the foregoing is presented as an example of what we must postulate if we assume that the phenomenon here presented is due to a selective excretion on the part of the kidney It seems to us easier to consider this phenomenon and the related one in the nephrosis syndrome as evidence of a defect in the intermediary sulphur metabolism

#### SUMMARY

1 The nitrogen and sulphur excretion of seven patients without renal edema were studied while these patients were on a diet constant as to nitrogen, sulphur, phosphorus, calories and water

2 It is demonstrated that there is a strong tendency for these patients to go into negative sulphur balance while remaining in positive nitrogen balance

3 The N S ratio in the urine of such patients tends to be low even when they are in positive sulphur balance

4 A contrast between the sulphur excretion in these patients and

those exhibiting the nephrosis syndrome suggests very strongly that there is a defect in the intermediary sulphur metabolism in patients with Bright's Disease.

#### CASE HISTORIES

*Case VI* Medical number 31861, a white male, aged 17, was admitted to the hospital on January 19, 1928, with the complaint of edema. Past history is unimportant except for one attack of tonsillitis followed by a tonsillectomy eight years ago. The present illness began the middle of December, 1927 with general malaise, followed on January 3 by a swelling of the right knee with pain but without fever. The knee was not red. There was considerable abdominal pain and three days later, he noticed a swelling of the face. There have been no other symptoms. Physical examination showed normal fundi, a diastolic murmur in the third left interspace and the associated signs of aortic insufficiency. Blood pressure, 130 systolic and 74 diastolic. There was edema of the symphysis and sacrum and a definite fluid wave was made out. Hands and legs both showed edema. Hemoglobin was 85 per cent, red blood count, 5,000,000, white blood count, 12,450. Urine showed a large trace of albumin, a very large number of red blood cells and many granular and hyaline casts. Phthalein excretion was 50 per cent, blood urea nitrogen, 31 mgm per 100 cc., Wassermann, negative. He was put on a low protein diet and by January 30, had lost 11.4 k.gm. Urine continued to show a large amount of albumin and many red cells. Blood pressure varied between 110 and 144 systolic, with a diastolic of 62 to 86. Phthalein excretion varied between 40 and 50 per cent. The red cells gradually cleared up, and he was discharged the middle of April.

*Case VII* Medical number 32195, a single white female of 24 years, entered the hospital on March 8, 1928 complaining of blurring of vision. She had had scarlet fever at 5, and 6 years ago following a severe cold, was admitted to the Massachusetts General Hospital complaining of edema of the ankles. She was there for 6 weeks, and since then has been free of symptoms. Three weeks ago, she first noted blurring vision following a cold in the chest, and at the same time had a sharply localized headache relieved by vomiting. The disturbance of vision has gradually improved. When seen in the hospital, blood pressure was 175 systolic, 125 diastolic. There was a marked albuminuric retinitis, heart was enlarged but regular. Hemoglobin, 80 per cent, red blood count, 4,620,000. The urine showed a trace of albumin, large number of casts of all kinds. Blood urea nitrogen was 8.4. There was practically no change in the urinary picture. The blood urea nitrogen never rose during this admission. Blood pressure fell somewhat, but rose just before discharge to 168 systolic, 122 diastolic. Phthalein excretion varied between 15 per cent shortly after admission to 32 per cent on discharge. She was discharged on April 23 and died September 10, 1929.

*Case VIII* Medical number 31781, a white female of 15 years, was admitted to the hospital on January 10, 1928. She was first seen in the Out-Door Department on December 30, with a hemoglobin of 50 per cent, the urine showing a large trace of albumin. She had always been well up to three years ago, when she noticed a sudden swelling and puffiness about both eyes and ankles. She had no respiratory infection. She went to the Boston City Hospital for a two weeks' stay, then left, but the edema returned 3 months later, in February, 1925 when she again went to the Boston City Hospital and remained there for 4 weeks. The edema returned 2 weeks after this admission, but gradually disappeared, and from September, 1926 to August, 1928 she was free of edema. At that time, 5 months before admission, she began to lose weight and it was noticed that she was getting paler. On admission, hemoglobin was 35 per cent, red blood count, 3,160,000, white blood count, 6300. Blood pressure, 120 systolic, 80 diastolic. Urine showed a large trace of albumin, a moderate number of red cells, but no casts. Phthalein excretion was 10 per cent, blood urea nitrogen 18. Blood pressure remained low. Phthalein excretion rose to 32 per cent on February 13. Red cells in the urine diminished and numerous hyaline and granular casts appeared. January 19 her hemoglobin had risen to 60 per cent but her red blood count was 2,980,000. She left the hospital on February 14.

*Case IX* Medical number 35349, a white female of 47 years, entered the hospital on August 5, 1929 complaining of edema. She had noticed this for 3 years becoming progressively worse. She had pain under her left breast at times, which did not radiate, but she had attacks of a sense of pressure in her chest, especially at night. She had become progressively weaker and had shown some loss of weight in the past 3 years. Lately swelling of the legs and ankles and headaches have developed, together with nausea and anorexia. Eleven months ago she noticed that her vision was beginning to fail. Physical examination showed very extensive retinal changes with sclerosis of the vessels, and exudate and hemorrhages. Heart was enlarged as was the aortic dullness, the rhythm was regular and no significant murmurs were heard. Blood pressure, 225 systolic, 140 diastolic. Urine showed small amount of albumin, many red cells and a great many casts of all kinds. She was digitalized. Hemoglobin was 62 per cent, red blood count, 4,700,000, blood urea nitrogen, 28 mgm and phthalein excretion, 40 per cent. By August 13 blood urea nitrogen had fallen to normal figures, blood pressure remained at about the admission level, phthalein excretion became stabilized at 25 per cent and edema had disappeared. The urine showed much the same picture except that fat appeared in the urine on August 23. She was discharged on September 3, blood urea nitrogen, 11, phthalein excretion, 25 per cent. She re-entered on December 18, 1929 with the typical picture of congestive heart failure. By December 24, renal insufficiency complicated the picture, the phthalein excretion being 5 per cent, blood urea nitrogen having risen to 25. She died in uremia January 7, 1930.

*Case X* Medical number 35649 A white male, aged 35, admitted to the hospital on September 28, 1929 complaining of headaches and vomiting spells He had been known to have "kidney trouble" since 1908, when his feet and legs were swollen for a month About a year ago, he noticed a feeling of ill health and an increase in his headaches, which he had had during this period, vomiting became more severe and of longer duration and has been almost constant in the last 4 months Eight years ago he had an attack of what was called infantile paralysis with almost complete bilateral facial paralysis with only slight symptoms in the arms and legs The facial paralysis lasted 6 months Physical examination showed only a slightly enlarged heart, hemorrhagic retinitis, blood pressure, 182 systolic, 110 diastolic, phthalein excretion was only a trace, hemoglobin, 30 per cent, red blood count, 3,200,000, blood urea nitrogen, 52, basal metabolic rate, minus three per cent He improved symptomatically Urine showed a low specific gravity, a slight trace of albumin and no formed elements of importance in the sediment On October 10, his phthalein excretion was less than 5 per cent, blood urea nitrogen, 32 mgm per 100 cc , hemoglobin, 50 per cent, red blood count, 3,750,000 On October 14, his blood urea nitrogen fell to 16 and his phthalein excretion rose to 10 per cent A week later blood urea nitrogen was again 53 mgm , the urine showed a slight trace of albumin and many coarsely granular casts His blood urea nitrogen varied between 50 and 27 and his phthalein excretion between 10 and 15 per cent. By the 31st of October, red blood cells had appeared in the sediment which persisted. On November 7, total blood protein was 7 6 per cent, albumin 5, globulin 2 6 He was discharged and readmitted in January, 1930 and died on April 22, 1930 He died in uremia with a blood urea nitrogen of 273 At autopsy the right kidney was contracted, weighing only 60 grams, and the left kidney was almost entirely replaced by a cystic degeneration

*Case XI* Medical number 35758, a white female, aged 16 years, admitted to the hospital on October 17, 1929, complaining of swelling of the ankles and puffiness of the eyes and headaches for 5 years The onset was sudden 5 years ago During the last 2 years, has had at intervals of 6 weeks to 2 months, attacks of nausea and vomiting Physical examination, at entrance, showed a hemoglobin of 80 per cent, red blood count, 5,200,000 There was a slight pitting edema of the ankles, heart was not enlarged, blood pressure, 150 systolic, 100 diastolic Urine showed many casts and slight trace of albumin Phthalein excretion, 50 per cent, blood urea nitrogen, 13 On October 24 basal metabolic rate was minus ten per cent, and on October 25, total protein was 7 8, albumin 5 6, globulin 2 2, cholesterol, 153 The urine continued to show slight trace to a large trace of albumin, with many casts Blood urea nitrogen ranged around 10 mgm per 100 cc. and blood pressure had fallen until at discharge it was 122 systolic, 88 diastolic Patient was discharged on November 15, 1929

*Case XII* Medical number 34368, a white male, aged 49 years, admitted to the hospital February 21, 1929 complaining of exhaustion and head pains During

the past year, the patient has felt generally tired but he has been able to rest and has been refreshed in the morning. Has been working extremely hard and has explained his general weakness on that basis. About the latter part of December, 1928 patient had a mild winter infection, following which he was extremely weak and was kept in bed 2 weeks. Since that time, his sleep has been interrupted and broken, and he has had extreme nocturia which he had attributed to nervousness. For the last 6 weeks there has been a persistent sense of weakness and generalized headache which has been present part of each day. There have been no other symptoms except that he has blown repeated clots of blood from the nostrils during the last week. No true epistaxis. Physical examination shows no enlargement of the heart, which is regular. Retinal vessels show edema of both nerve-heads and the retinal vessels are very tortuous, veins dilated, and some recent hemorrhages are seen. Blood pressure, 236 systolic, 156 diastolic. Gradually improved on rest in bed, blood pressure at one time being as low as 200 systolic, 128 diastolic. The edema of the retinae subsided and his headaches diminished in intensity. Urine constantly showed a trace to a large trace of albumin, and some fixation of specific gravity. Phthalein excretion on admission was 12 per cent. There were constantly numerous casts of all sorts in his urine. Hemoglobin was 80 per cent, red blood count, 4,900,000 on March 19. On March 1, total protein was 4.2, albumin 3.1, globulin 1.1. Blood urea on March 12 was 15 mgm, blood uric acid 6.6. Blood creatinin on March 15 was 3 mgm, blood uric acid 5.6. On March 19, the blood nonprotein nitrogen was 39 mgm per 100 cc. Patient was discharged on March 20. Blood pressure had returned to level of 224 systolic, 150 diastolic. He died April 23, 1929.

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## ICTERUS NEONATORUM

### IV THE RÔLE OF THE PLACENTA IN VISIBLE ICTERUS NEONATORUM

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In three previous publications on icterus neonatorum one of us (1) has demonstrated that the polycythaemia in the new-born is due probably to an anoxaemia of the foetus in utero and is an expression of compensation by the active haematopoietic system. After birth the excess of red blood corpuscles is rapidly destroyed, since they are no longer required for the maintenance of oxygenation. The rapid blood destruction leads to an increase of the bilirubin in the serum and jaundice.

We concluded that "icterus neonatorum is a physiological condition, which is the result of a postnatal readjustment from an environment requiring the presence of polycythaemia for the maintenance of oxygenation to one in which no such extraordinary measures are necessary. Icterus is present in all infants, visibility being only a matter of degree."

Although we contend that visible jaundice is but the final result of the conditions existing in the new-born infant, it may be that there are certain other factors which in some cases will make visible jaundice a more prominent feature.

The natural weakness of the placenta as a respiratory organ, in comparison with the lung, and its tendency to marked degenerative changes, suggested to us a possible source for an intensification of the degree of foetal anoxaemia, polycythaemia and resultant icterus.

We therefore studied the relation of the condition of the placenta to the degree of jaundice in 85 consecutive new born infants.

The method of procedure was as follows. As soon as the infant was born and before delivery of the placenta a sample of umbilical blood was taken. A complete morphological study of the erythrocytes was

made and the bilirubin content was estimated by the Van den Bergh reaction. These studies were repeated daily in the infant for one week and the appearance of visible jaundice was watched for carefully. Sections of the placenta were taken in each case and thoroughly studied.

The degree of jaundice as well as the changes in the placenta were classified in three groups—mild, moderate and severe.

TABLE 1  
*Showing number of subjects in each group*

	Placental changes				
	None	Mild	Moderate	Severe	Total
Jaundice not visible	31	9	0	0	40
Jaundice visible	14	9	12	10	45
Total	45	18	12	10	85

TABLE 2  
*Relation of polycythaemia and bilirubinaemia at birth to the condition of the placenta in 85 cases*

	Placental changes			
	None	Mild	Moderate	Severe
Jaundice not visible				
Red blood cells, millions	5 21	5 25		
Hemoglobin, per cent	102	102		
Van den Bergh units	2 8	2 8		
Jaundice visible				
Red blood cells, millions	6 20	5 90	6 55	7 11
Hemoglobin, per cent	116	110	118	122
Van den Bergh units	5 5	4 2	5 7	6 8

Placentae classified as "mild degenerative changes" showed no macroscopic pathology. Microscopic sections showed numerous partly or completely hyalinized villi affording evidence of advancing senile change in the vascular tree. Those classified as "moderate degenerative changes" showed macroscopically whitish infarcted areas or scattered soft sequestra of tissue and microscopically either large

hyalinized areas of villi, sometimes with productive changes in the chorionic membrane, or irregular areas of intervillous congestion, evidence of early maternal infarction. Those described as "severe degenerative changes" showed macroscopically relatively thick, greyish friable placentae with large whitish or infarcted areas or large soft sequestra as evidence of maternal infarction. Microscopic examination substantiated this.

In 40 out of the 85 cases studied, changes in the placenta were found. In 31 of these 40 cases visible jaundice was present, that is in 78 per cent of these cases with changes in the placenta, while 9 cases or 22 per cent did not show any visible jaundice. The changes of the placenta in these 9 cases were, however, all in the group of "mild changes".

In 45 of the 85 cases, visible jaundice was present, 31 of them had changes in the placenta, that is 69 per cent of all the cases exhibiting visible jaundice, while 14 cases or 31 per cent did not show any placental changes.

Table 1 shows the relation of jaundice to the condition of the placenta.

The relation of the polycythaemia and bilirubinaemia is demonstrated in table 2.

From this table one can see that changes of the placenta would appear to lead to an increase in the foetal polycythaemia as well as the foetal bilirubinaemia. That is especially the case where the placental changes are well marked, as in the group of the moderate and severe changes.

#### DISCUSSION OF FINDINGS

From the above findings it can be seen that changes of the placenta, if marked enough, lead to an exaggeration of the prenatal conditions, that is an increase in the foetal polycythaemia and bilirubinaemia or, in other words, marked changes of the placenta diminish its respiratory ability, intensifying the already existing foetal anoxaemia, which in turn calls forth for an increase in the compensatory polycythaemia. In the process of adaptation to postnatal conditions in these cases it is quite obvious that the greater amount of red blood corpuscles offered for destruction will cause a deepening of the postnatal increase of the bilirubinaemia and eventually lead to visibility of the jaundice.

The 14 cases of visible jaundice without changes in the placenta, on the other hand, show that placental changes, although an important factor in intensifying the degree of icterus neonatorum, are not responsible in every case Other factors as yet unknown may come into play

#### CONCLUSION

Pathological changes in the placenta are associated with an increase in the foetal polycythaemia, with a subsequent increase in the degree of icterus neonatorum, making its visibility a more marked feature

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## STREPTOCOCCAL AGGLUTININS IN CHRONIC INFECTIOUS ARTHRITIS<sup>1</sup>

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The development of specific agglutinins in the course of acute or chronic infections has long been recognized as one of the most constant and characteristic of the phenomena which follow bacterial invasion of the animal organism. Indeed, this biologic reaction is such a well nigh universal concomitant of infection that it has come into wide use clinically as a diagnostic measure.

In a previous paper, the writers (1), in collaboration with R. L. Cecil, have reported the isolation of streptococci from the blood and joints of a high percentage of patients with chronic infectious arthritis. Eighty three and three-tenths per cent of the streptococcal strains thus isolated were culturally identical and were designated as "typical strains." Somewhat to our surprise, these "typical strains" were cross agglutinated by homologous immune rabbit serums. These observations naturally led to a search for streptococcal agglutinins in the serums of patients with chronic infectious arthritis, and the present communication embraces the results obtained in this investigation.

### METHODS

*1. Agglutination tests.* The antigen consisted of a 24-hour growth of a strain of streptococcus in beef heart infusion broth (pH 7.6). Five tenths cubic centimeter of the bacterial antigen was added in turn to a series of agglutination tubes each containing 0.5 cc. of the diluted serum. The dilutions were made in plain broth and the final

<sup>1</sup> This investigation was supported in part by the Committee for the Encouragement of Medical Research.

dilutions were 1 20, 1 40, 1 80, 1 160, 1 320, 1 640, 1 1,280, 1 2,560 and 1 5,120. The controls consisted of normal serum and broth without serum. The tubes were heated in a water bath at 56°C for 2 hours, placed in the refrigerator overnight and read the following morning. The highest dilution in which definite agglutination was observed was recorded as the agglutination titer. Most of the agglutination tests were carried out by the operator without any knowledge of the identity of the serums.

At times there was a tendency for the cultures to become granular. This difficulty was overcome by successive passages of the streptococcal strain through blood broth for several days before finally transferring it to plain broth.

*2 Absorption tests.* Five tenths cubic centimeter of a 1 5 dilution of the serum in broth was added to the sediment from 150 cc of a 24-hour broth culture of the streptococcus. The mixture was thoroughly shaken and placed for 2 hours in a water bath at 37°C where it received further shaking at frequent intervals. The mixture was then placed in the refrigerator overnight, and in the morning it was centrifugalized and the supernatant fluid removed. Agglutination tests were then carried out with the absorbed serum, using the same dilutions as in the original reactions. Control tests were carried out with unabsorbed serum. All the tubes were then heated for 2 hours in a water bath at 56°C. Readings were made the following morning.

#### *Chronic infectious arthritis*

The type of patient investigated presented the syndrome generally known as chronic infectious arthritis or rheumatoid arthritis, namely, a chronic progressive polyarthritis with swelling and often with some deformity and ankylosis of the joints affected. The fusiform swelling of the fingers is a characteristic physical sign.

Agglutination reactions were carried out with the serums from 110 cases of chronic infectious arthritis against "typical strain" streptococci which had previously been recovered from the blood or joints of patients with this disease. Various "typical strains," but especially strain AB13, were used in these studies. Of the 110 serums examined, 103, or 93.6 per cent, showed definite agglutination with "typical"

"strain" streptococci to a titer of 1,640 or more (table 1, chart 1). Two of the remaining 7 serums were from patients who had had arthritic symptoms for only 8 and 60 days respectively.

### Controls

Agglutination reactions against "typical strain" streptococci were carried out in the same way with the serums of 218 normal persons or patients with other diseases. It was considered advisable to divide the controls into several groups, namely, rheumatic fever, degenerative

TABLE I  
Titers of agglutination reactions of "typical strain" with patients' serums

Disease	Dilutions of serum*									
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
Chronic Infectious arthritis	110	1	1	1	1	2	17	21	19	21
Acute rheumatic fever	70	51	3	12	10	2	1	0	0	0
Degenerative arthritis	16	9	3	2	2	0	0	0	0	0
Acute tonsillitis acute bronchitis	8	0	0	0	4	1	1	0	1	0
Other febrile diseases	27	13	2	3	3	2	1	1	0	0
Other non febrile diseases	41	22	4	6	5	2	0	1	1	0
Normal people	47	37	1	4	4	1	0	0	0	0
Healthy rabbits	6	0	0	0	0	0	0	0	0	0

\* The numerals under the various dilutions indicate the number of cases with the limit of agglutination at that dilution.

arthritis, acute tonsillitis and acute bronchitis, other febrile diseases, other non febrile diseases and normal individuals. The serums of 6 healthy rabbits were also studied for streptococcal agglutinins.

The serums of 79 patients with acute rheumatic fever were examined for specific agglutinins against the "typical strain" of streptococcus. Of these, 51, or 64 per cent, failed to show any agglutination, while none of them gave an agglutination to a titer higher than 1:320 (table 1, chart 1).

The next control group consisted of 16 cases of degenerative arthritis. Care was taken to select patients who showed degenerative

arthritic changes only, none of the joints showed soft tissue swelling and x-rays revealed the characteristic hypertrophic changes in the bones. Of these 16 cases, 9, or 56.3 per cent, showed no agglutination whatever with "typical strain" streptococci, the remaining 7, gave very weakly positive reactions, 3 at a titer of 1:20, 2 at a titer of 1:40,

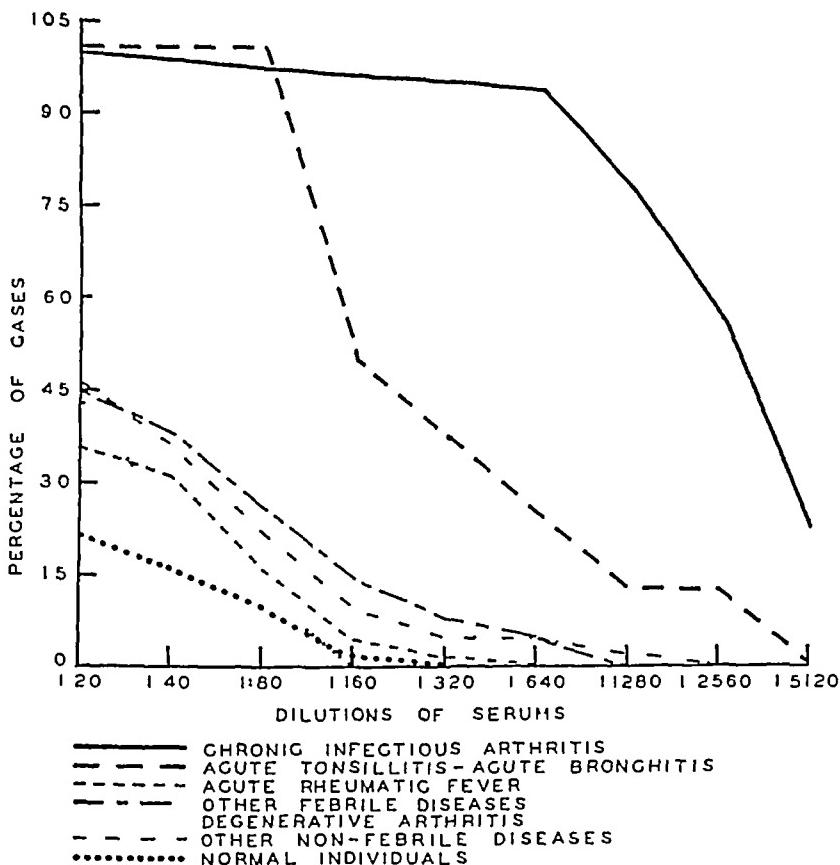


CHART 1 TOTAL AGGLUTINATIONS WITH PATIENTS' SERUMS AGAINST "TYPICAL STRAINS"

and 2 at a titer of 1:80 (table 1, chart 1). Probably these weakly positive reactions have no significance. In view of the fact that, in our experience, streptococci have never been recovered from the blood and joints of patients with degenerative arthritis, the absence of streptococcal agglutinins in the latter group of cases is quite consistent,

and in striking contrast to the strong reactions obtained in cases of chronic infectious arthritis.

An interesting control group consisted of 8 patients suffering from acute tonsillitis or acute bronchitis. The serum of one of these, a case of acute bronchitis, agglutinated with "typical strains" to a titer of 1 2,560, while the lowest agglutination titer recorded for this group of patients was 1 80 (table 1, chart 1). The serums of some of these patients gave agglutination reactions comparable to the serums of patients with chronic infectious arthritis, although the average titer for the upper respiratory infections was considerably lower. These observations were particularly interesting in view of the frequent association of infections of the upper respiratory tract with chronic infectious arthritis.

Twenty-seven patients with febrile conditions other than rheumatic fever and acute upper respiratory infections were tested for specific agglutinins against "typical strains" of streptococci. This group included cases of jaundice, pleurisy with effusion, acute nephritis, and pneumonia. The serum of only one of these, a patient with acute nephritis, gave an agglutination titer as high as 1 640, 15, or 55 per cent, showed no agglutination whatever, while the remaining cases showed only weakly positive reactions (table 1, chart 1). The negative or weakly positive results obtained from this group of controls would appear to indicate that the agglutination observed in patients with chronic infectious arthritis cannot be looked upon as a physical phenomenon indicative of infection in general but as a specific reaction to streptococcus infection.

The serum of 41 patients with non febrile diseases other than arthritis were studied in the same way. This group consisted of patients with new growths, hypertension, and other non febrile diseases. Only 2 of the 41 cases gave an agglutination of 1 640 or higher, 22, or 53 7 per cent, showed no agglutination, while the remainder gave positive agglutinations only in the lower dilutions (table 1, chart 1).

The serums of 47 normal persons were studied in the same manner, 37, or 78 7 per cent, failed to show any agglutination whatever with the "typical strain" of streptococcus. The highest agglutination titer recorded for this group was 1 160, a titer which has little, if any, significance (table 1, chart 1).

The serums of 6 healthy rabbits failed to show any agglutination with "typical strain" cultures

*Chronic polyarthritis following rheumatic fever*

During the course of this investigation, the serums of 3 patients with chronic polyarthritis following rheumatic fever were studied for the presence of streptococcal agglutinins. All 3 patients gave histories of one or more attacks of rheumatic fever. Clinically these three cases presented a picture indistinguishable from that of chronic infectious arthritis except for the presence of endocarditis presumably rheumatic. The agglutination titer with "typical strain" cultures was 1:80 in one case, and entirely negative in the other two cases. As will be shown in a subsequent communication, these patients showed strongly positive agglutination reactions with a streptococcus recovered from the blood of a patient with acute rheumatic fever.

*Disappearance of streptococcal agglutinins after recovery from chronic infectious arthritis*

In 3 patients who recovered from chronic infectious arthritis, a comparison of the agglutination reactions with the "typical strain" of streptococcus during the course of the disease was made with the reactions in the same patients after recovery from the infection.

A brief summary of the 3 cases is presented.

M P, female, aged 27, chronic infectious arthritis of 2 years' duration. Agglutination titer with "typical strain" 1:1,280. Tonsillectomy performed, following which patient became free from symptoms. Three weeks after tonsillectomy the agglutination titer was 1:320, one year after, the agglutination test was entirely negative.

M D, female, aged 30, chronic infectious arthritis of 3 months' duration, agglutination titer with "typical strain" 1:1,280. Tonsillectomy performed, following which patient completely recovered from arthritis. Three weeks after tonsillectomy agglutination titer was 1:40, 3 months after, the agglutination titer was entirely negative.

S W, male, aged 50, chronic infectious arthritis of 3 months' duration, agglutination titer with "typical strain" 1:2,560. Tonsillectomy performed, following which patient became free from active disease. Three months after tonsillectomy agglutination test was entirely negative.

*Stability of the streptococcal agglutinins*

Agglutination reactions were performed on the serums of patients suffering from chronic infectious arthritis with "typical strains," at approximately weekly intervals up to 90 days, in order to determine whether or not the serums retained their agglutinins for these streptococci over an appreciable length of time. During this period the serums were kept in a refrigerator at almost freezing temperature. It was found that the agglutinating property did not diminish perceptibly during the entire 90 days.

*Streptococcal agglutinins in joint fluids*

Agglutination reactions with "typical strains" were carried out on the joint fluids of 3 patients with chronic infectious arthritis. The technic for testing the joint fluids was similar in every respect to that employed for serums. The agglutination titers were surprisingly high, 1 640, 1 2,560, and 1 5,120, respectively. The only control joint fluids available were from 4 patients with acute rheumatic fever. The agglutination reaction in each of the controls was entirely negative.

*Specificity of the agglutination reactions*

It has already been shown that the serums of patients with chronic infectious arthritis agglutinate to a high titer with "typical strains." In order to check the specificity of these reactions, agglutination tests were performed with the serums of patients with chronic infectious arthritis against various pathogenic organisms. This work was controlled with the serums of patients having rheumatic fever, sub acute bacterial endocarditis, and acute tonsillitis and with the serums of normal individuals (table 2).

As was expected, "typical strain" AB13 showed the usual high titers of agglutination with the serums of patients with chronic infectious arthritis. Much to our surprise, a *Streptococcus hemolyticus* from a case of scarlet fever and a *Streptococcus hemolyticus* from a case of erysipelas gave agglutination titers with the arthritic serums comparable to that given by the "typical strain" streptococcus, although on the average the end reactions were at lower dilutions. A rheumatic fever strain

TABLE 2  
Titers of agglutination reactions of various pathogenic organisms with the serums of patients with chronic infectious arthritis and of controls

Patient	Disease	Bacterial antigens										
		AB13	Sc. Fv	Erysip.	RB5	Pn 1	Pn 2	Pn 3	S. albus	Strep. th.	B. col.	S. aur.
1	Chronic infectious arthritis	1 5,120	1 5,120	1 2,560	1 40	0	0	0	0	0	0	1 80
2	Chronic infectious arthritis	1 5,120	1 640	1 2,560	1 160	0	0	1 320	1 80	1 40	0	1 80
3	Chronic infectious arthritis	1 5,120	1 2,560	1 1,280	1 40	0	0	0	0	0	0	1 80
4	Chronic infectious arthritis	1 2,560	1 2,560	1 5,120	1 160	0	0	0	0	0	0	1 320
5	Chronic infectious arthritis	1 1,280	1 320	1 1,280	0	0	0	0	0	0	0	0
6	Chronic infectious arthritis	1 5,120	1 320	1 640	1 320	0	0	0	0	0	0	1 80
7	Chronic infectious arthritis	1 1,280	1 1,280	1 1,280	0	0	0	0	0	0	0	0
8	Chronic infectious arthritis	1 1,280	1 1,280	1 640	0	0	0	0	0	0	0	1 80
9	Acute rheumatic fever	1 80	0	0	1 320	0	0	0	0	0	0	1 2,560
10	Acute rheumatic fever	0	0	0	1 1,280	0	0	0	0	0	0	0
11	Acute rheumatic fever	1 80	1 80	0	1 320	0	0	0	0	0	0	1 640
12	Acute rheumatic fever	0	0	0	1 160	0	0	0	0	0	0	0
13	Acute rheumatic fever	0	0	0	1 320	0	0	0	0	0	0	0
14	Subacute bacterial endocarditis	1 80	0	0	1 640	0	0	0	0	0	0	0
15	Subacute bacterial endocarditis	0	0	0	1 1,280	0	0	0	0	0	0	0
16	Acute tonsillitis	0	0	0	1 160	0	0	0	0	0	0	0
17	Acute tonsillitis	1 160	0	0	1 40	0	1 80	0	0	0	0	0
18	Normal person	0	0	0	0	0	0	0	0	1 2,560	0	1 80
19	Normal person	0	1 2,560	0	0	0	0	0	0	0	0	0
20	Normal person	0	0	0	0	0	0	0	0	0	0	1 160
21	Normal person	0	0	0	1 20	0	0	0	0	1 80	0	1 320
22	Normal person	1 40	0	0	1 40	0	0	0	0	0	0	0
23	Normal person	1 20	0	0	1 80	0	0	0	0	0	0	0

AB13 = "typical strain" streptococcus, Sc. Fv = scarlet fever streptococcus, Erysip. = erysipelas streptococcus, RB5 = rheumatic fever streptococcus, Pn. 1 = pneumococcus type 1, Pn. 2 = pneumococcus type 2, Pn. 3 = pneumococcus type 3, S. albus = *Staphylococcus albus*, Strep. th. = hemolytic streptococcus from the throat, B. col. = *Bacillus coli*, S. aur. = *Staphylococcus aureus*.

of *Streptococcus viridans* (RB5) showed weakly positive reactions with arthritic serums, but the titers were only slightly higher than those given by this streptococcus against normal serums. With the exception of the streptococci from chronic infectious arthritis, scarlet fever, and erysipelas, the various bacterial antigens tested showed only insignificant reactions. The serums from patients with other infections and from normal persons gave agglutination reactions of little importance except in the case of the rheumatic fever strain RB5 which showed strong agglutination reactions with the serums from cases of rheumatic fever and subacute bacterial endocarditis.

The agglutination tests with the serums of patients having chronic infectious arthritis against "typical strain" AB13, the scarlet fever streptococcus and the erysipelas streptococcus were controlled by direct absorption tests. These were performed by absorbing each of the arthritic serums with cultures of each of the three hemolytic streptococci. Agglutination tests were then carried out with the absorbed serums against their absorbing strains. Unabsorbed serums were used as controls. Complete or almost complete absorption was effected in each case.

The nature of the agglutinins in the serums of patients with chronic infectious arthritis was further studied by cross absorption reactions with the serums of 2 patients with chronic infectious arthritis and the serum of a rabbit which had been previously immunized with the "typical strain" AB13. Each of the three serums was in turn subjected to absorption tests by the "typical strain" streptococcus AB13, the scarlet fever streptococcus and the erysipelas streptococcus. Agglutination tests were then carried out with the cultures of each of the three hemolytic streptococci on each of the absorbed serums. Complete or almost complete absorption was effected in each case (table 3). The cross absorption tests indicate the antigenic relationship of "typical strain" streptococci to the hemolytic streptococci from cases of scarlet fever and erysipelas.

Finally, it seemed important to perform additional agglutination tests with arthritic serums against hemolytic streptococci from patients who gave no history of arthritis, scarlet fever, or erysipelas. Eight such strains were selected (table 4). Two of these strains, both recovered from throat cultures, showed an agglutination titer as high

TABLE 3

*Agglutination and absorption reactions with the serums of two patients with chronic infectious arthritis and of one immune rabbit against hemolytic streptococci from chronic infectious arthritis, scarlet fever and erysipelas*

Serums	Absorbing strains	Bacterial antigens		
		AB13	S F 1	E 1
R W	Unabsorbed	1 1,280	1 640	1 320
	AB13	0	0	0
	S F 1	0	0	0
	E 1	1 20	0	0
L M	Unabsorbed	1 5,120	1 640	1 640
	AB13	0	0	0
	S F 1	0	0	0
	E 1	1 20	0	0
Rabbit 177	Unabsorbed	1 5,120	1 640	1 1,280
	AB13	0	1 20	0
	S F 1	0	0	1 20
	E 1	0	0	1 20

AB13 = "typical strain" streptococcus, S F 1 = scarlet fever streptococcus, E 1 = erysipelas streptococcus, R W and L M = patients with chronic infectious arthritis, rabbit 177 = rabbit immunized against strain AB13

TABLE 4

*Titer of agglutination reactions of various hemolytic streptococci with the serums of patients with chronic infectious arthritis*

Patient	AB13	Sc Fv	Erysip	Throat H.S 1	Throat H.S 2	Throat H.S 3	Throat H.S 4	Tonsil	Tooth	Stool	Gastric ulcer
24	1 5,120	1 1,280	1 160	1 80	1 20	1 80	0	0	0	1 80	1 20
25	1 640	1 1,280	1 160	0	0	1 80	0	1 160	0	0	1 20
26	1 640	1 640	1 320	0	0	0	0	0	1 20	0	1 40
27	1 5,120	1 640	1 1,280	0	1 80	0	0	0	0	1 20	0
28	1 5,120	1 2,560	1 2,560	1 640	0	1 640	0	1 160	0	0	0
29	1 5,120	1 320	1 320	1 160	0	1 80	0	1 80	0	0	0
30	1 640	1 160	1 160	1 20	0	0	0	0	0	0	0

AB13 = "typical strain" streptococcus, Sc Fv = scarlet fever streptococcus, Throat = hemolytic streptococci isolated from the throats of various individuals, Tonsil = hemolytic streptococcus isolated from an excised tonsil, Tooth = hemolytic streptococcus isolated from a root canal of a tooth, Stool = hemolytic streptococcus recovered from a stool specimen, Gastric ulcer = alpha prime type of streptococcus isolated from a gastric ulcer by E W Saunders (2)

as 1:640 with the serum of one of the patients with chronic infectious arthritis, while the others showed slight or no agglutination. It seems probable that if the series had been larger, several other strains would have agglutinated well with the serums of patients with chronic infectious arthritis. The observations from these agglutinations were quite compatible with those reported by Stevens and Dochez (3), who performed agglutination tests with immune serums against scarlet fever, erysipelas, and pyogenic streptococci, and found that some of the pyogenic streptococci had antigenic properties in common with the hemolytic streptococci from scarlet fever and erysipelas.

#### SUMMARY AND DISCUSSION

The serums of patients with chronic infectious arthritis usually gave strong agglutination reactions with "typical strain" streptococci (93.6 per cent in this series). This phenomenon is not observed with control serums with the possible exception of the serums from patients with acute tonsillitis and acute bronchitis. The close association of upper respiratory infection with chronic infectious arthritis has already been mentioned.

In marked contrast to these strong agglutinative properties in the serums of patients with chronic infectious arthritis is the slight or negative agglutinin content of the serums of patients with degenerative arthritis. This biologic distinction between the two types of arthritis offers strong evidence in support of the theory of their diverse etiologies—indeed, the contrast is so definite that it might well be used clinically in the differential diagnosis of the two most common chronic infections of the joints.

The serums of 3 patients with chronic polyarthritis following rheumatic fever failed to give positive agglutination reactions with "typical strains." This would seem to indicate that chronic polyarthritis following rheumatic fever and primary chronic infectious arthritis are not etiologically identical, although clinically they present similar pictures.

Three patients whose serums gave strong agglutination reactions during the course of the disease were found to have completely lost their agglutinins following recovery. In the opinion of the writers,

these observations lend strong support to the theory that the "typical strain" streptococcus is an important etiologic factor in chronic infectious arthritis.

The demonstration of streptococcal agglutinins in the joint fluids of 3 cases of chronic infectious arthritis was particularly interesting in view of the fact that in a previous study we had succeeded in recovering "typical strains" of streptococci in joint cultures from such patients. This is analogous to the high agglutinin content of empyema fluid in pneumococcal pneumonia.

A *Streptococcus hemolyticus* from a case of scarlet fever and a *Streptococcus hemolyticus* from a case of erysipelas have been agglutinated by the serums of arthritic patients to almost as high a titer as have "typical strain" streptococci. Stevens and Dochez (3) have shown that scarlet fever streptococci and erysipelas streptococci, as well as the individual strains from each disease, are not all biologically identical, but that those strains that are not identical usually have antigenic properties in common. It would appear probable, then, that if a larger number of strains from scarlet fever and erysipelas patients had been studied in this investigation, a certain number of them would have given strongly positive agglutination reactions with arthritic patients' serums, while others would have given weakly positive or negative agglutination reactions.

Two hemolytic streptococci from the throats of patients free from any evidence of arthritis, scarlet fever, or erysipelas gave agglutination reactions as high as 1:640 with the serum from a patient with chronic infectious arthritis, while the other hemolytic streptococci gave little or no agglutination. It seems reasonable to conclude that the "typical strain" streptococci have antigenic properties in common with the hemolytic streptococci from patients with scarlet fever and erysipelas, as well as with some hemolytic streptococci from other sources. Such an admission, however, in no wise weakens the confidence of the writers that the agglutinins present in the serums of patients with chronic infectious arthritis are specific agglutinins differing in no essential respect from the specific agglutinins which accompany infections with the typhoid bacillus, the pneumococcus, or any other pathogenic micro-organisms.

## CONCLUSIONS

- 1 The serums of patients with chronic infectious arthritis usually give a strong specific agglutination reaction with "typical strains" of streptococci recoverable from the blood and joints of patients with this disease. Control serums do not show this reaction.
- 2 Chronic infectious arthritis can be differentiated from degenerative arthritis and from chronic polyarthritis following rheumatic fever by the agglutination reaction. The agglutination reactions suggest different etiologies for the three forms of arthritis.
- 3 A close antigenic relationship between "typical strain" streptococci and the hemolytic streptococci from scarlet fever and erysipelas is established.

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## STREPTOCOCCAL AGGLUTININS IN RHEUMATIC FEVER<sup>1</sup>

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Although considerable evidence has accumulated that rheumatic fever is a streptococcal infection, attempts to demonstrate specific streptococcal agglutinins in the serums of patients with this disease have met with only a moderate degree of success Tunnicliff (1) performed agglutination reactions with the serums of twelve rheumatic fever patients against strains of *Micrococcus rheumaticus* and *Streptococcus pyogenes*, and demonstrated agglutinins in small amounts in seven instances Swift and Kinsella (2) failed to find any streptococcal agglutinins in the serums of five patients with rheumatic fever against streptococci which they had recovered from their respective blood cultures Clawson (3) working with strains of *Streptococcus viridans* isolated from cases of rheumatic fever, found agglutinins in four out of the five serums examined Small (4) demonstrated agglutinins for the so-called "streptococcus cardioarthritidis" in the serums of ten patients with rheumatic fever

In a previous paper the writers, in collaboration with Cecil (5), have reported the isolation of streptococci from the blood and joints of a high percentage of patients with rheumatic fever These strains, with one exception, belonged to the general group of non hemolytic streptococci<sup>2</sup> Cross agglutination and absorption tests performed with immune rabbit serums indicated that while these streptococci

<sup>1</sup> This investigation was supported in part by the Committee for the Encouragement of Medical Research

<sup>2</sup> There is some debate among bacteriologists as to whether the streptococci which we have isolated from cases of rheumatic fever belong to the viridans or indifferent group For this reason it seems appropriate to use the more general term, "non hemolytic streptococci"



or 66.7 per cent, agglutinated at very low titers (1:20 to 1:160). Only 4 of the 45 serums produced agglutinations at a titer of 1:320.

The next control group consisted of 16 patients with degenerative (hypertrophic) arthritis. In this group the highest agglutination titer recorded was 1:160.

The serums of 8 patients with acute tonsillitis or acute bronchitis were also tested for streptococcal agglutinins. Three gave no agglutination whatever, while the highest recorded was 1:80. In view of the frequency with which tonsillitis is associated with rheumatic

TABLE I  
*Titers of agglutinations of strain RB5 with patients' serums*

Disease	Total number of cases	Number of cases giving agglutination	Dilutions of serum*								
			1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	
Rheumatic fever	59	1	0	1	5	6	7	19	5	10	5
Chronic infectious arthritis	45	11	3	9	9	9	4	0	0	0	0
Degenerative arthritis	16	7	3	3	2	1	0	0	0	0	0
Acute tonsillitis, acute bronchitis	8	3	0	4	1	0	0	0	0	0	0
Other febrile diseases	23	8	0	8	1	2	1	3	0	0	0
Other non febrile diseases	30	10	2	3	6	3	3	2	1	0	0
Normal persons	40	16	6	4	10	4	0	0	0	0	0
Healthy rabbits	6	4	0	2	0	0	0	0	0	0	0

\* The numerals under the various dilutions indicate the number of cases with the limit of agglutination at that dilution.

fever, the results of the agglutination reactions of this group were unexpected.

The next control group consisted of 23 serums from patients with various febrile diseases other than rheumatic fever and subacute bacterial endocarditis, of which only 4 gave agglutination reactions at a titer of 1:320 or higher, the remaining 19 gave slight or negative reactions.

In the study of the serums of 30 patients with non-febrile diseases other than arthritis 24, or 80 per cent, failed to produce any agglutination in dilutions above 1:160, 3 showed weakly positive reactions, agglutinating to a titer of 1:320, one serum from a case of exophthal-

in general showed a tendency to fall into a number of biologic groups, one strain, RB5, had antigenic properties in common with the majority of these rheumatic fever streptococci. By reason of this fact, it occurred to us that this strain (RB5) would be particularly appropriate as an antigen in testing for streptococcal agglutinins in the serums of patients with rheumatic fever. The present communication embraces the results obtained in this investigation.

#### METHODS

The technic for carrying out the agglutination reactions was identical with that reported in the preceding study of streptococcal agglutinins in the serums of patients with chronic infectious arthritis.

#### *Rheumatic fever*

The patients selected for study presented definite signs of acute rheumatic infection. The majority of the cases were adults with rheumatic polyarthritis but several were children in whom signs of rheumatic infection consisted of fever, leucocytosis, and rheumatic heart disease.

Agglutination reactions were carried out with the serums from 59 patients with rheumatic fever against strain RB5. Of the 59 serums examined 46, or 78 per cent, showed definite agglutination to a titer of 1:320 or more, 39, or 66 per cent, to a titer of 1:640 or more, the serums of the remaining 13 patients gave only slight or negative agglutination reactions (table 1, chart 1).

#### *Controls*

Agglutination reactions against strain RB5 were carried out with the serums of 162 normal persons or patients with other diseases (table 1, chart 1). It was considered advisable to divide the controls into several groups, namely, chronic infectious arthritis, degenerative arthritis, acute tonsillitis and bronchitis, other febrile diseases, other non-febrile diseases and normal individuals. The serums from 6 healthy rabbits were also studied for streptococcal agglutinins.

The serums of 45 patients with chronic infectious arthritis were examined for agglutinins with the streptococcal strain RB5. Of these, 11, or 24.4 per cent, failed to show any agglutination whatever, 30,

*Chronic polyarthritis following rheumatic fever*

The serums from 3 patients with chronic polyarthritis following rheumatic fever were also studied for streptococcal agglutinins with strain RB5. The titers were 1 640, 1 320, and 1 320, respectively. Control agglutination tests carried out with the same serums against "typical strain" cultures of streptococci from cases of primary chronic infectious arthritis were practically negative.

*Subacute bacterial endocarditis*

One of the most interesting phases of this investigation was the study of the serums of 6 patients with subacute bacterial endocarditis

TABLE 2  
*Titers of agglutinations with serums of patients with subacute bacterial endocarditis*

Patient	Blood culture	Bacterial antigens	
		RB5	AB13†
1	<i>Streptococcus viridans</i>	1 320	1 40
2	<i>Streptococcus viridans</i>	1 640	0
3	<i>Streptococcus viridans</i>	1 1,280	0
4	<i>Streptococcus viridans</i>	1 5,120	0
5		1 640	0
6	<i>Streptococcus viridans</i>	1 1,280	1 80

\* RB5 strain of streptococcus recovered from the blood of a patient with rheumatic fever

† AB13 "typical strain" streptococcus recovered from the blood of a patient with chronic infectious arthritis.

Each of these patients presented the typical clinical picture of the disease, and blood cultures from 5 of the 6 cases yielded *streptococcus viridans*. In table 2 the agglutination titers of the serums of these 6 patients against strain RB5 and against the "typical strain" of chronic infectious arthritis (AB13) are presented. Each of the serums produced an agglutination to a high titer with the rheumatic fever strain, but showed little or no agglutination with strain AB13.

*Diminution of agglutinin content in serums following recovery*

In 4 patients with acute rheumatic fever, the agglutinin content of their serums was studied during the convalescent stage of the disease

mic goiter agglutinated strain RB5 to a titer of 1 640, 2 serums from patients with auricular fibrillation agglutinated to a titer of 1 1280 and 1 640, respectively

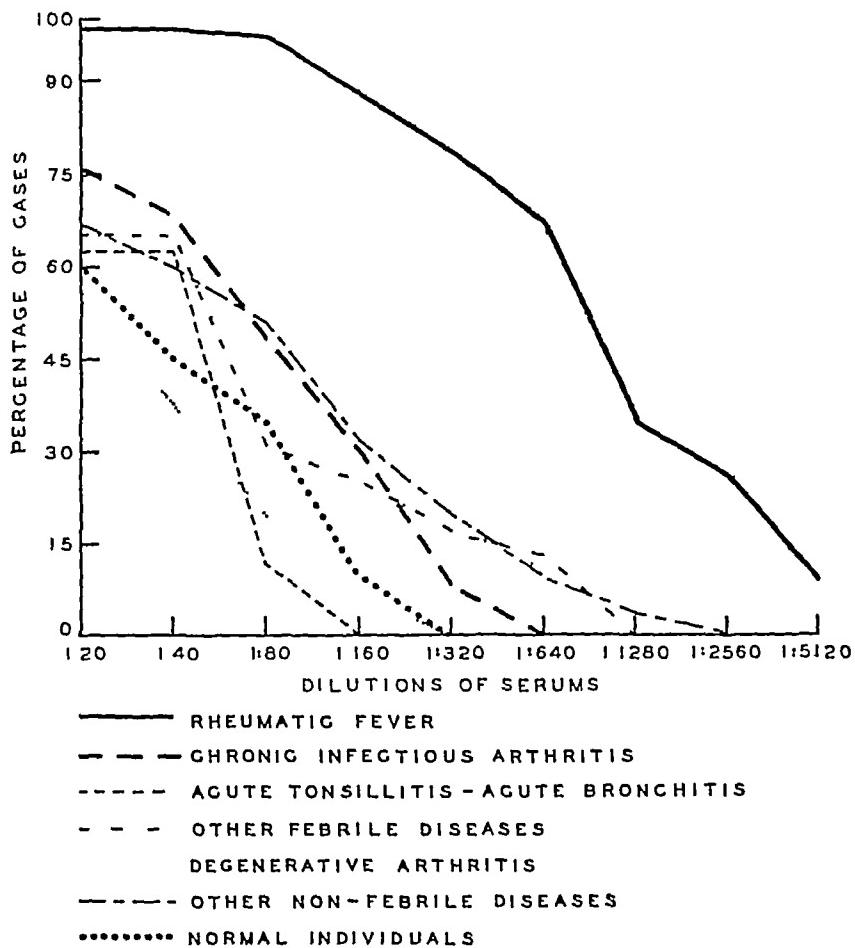


Chart 1 TOTAL AGGLUTINATIONS WITH PATIENTS' SERUMS AGAINST STRAIN RB5

The serums from 40 normal individuals failed to produce any agglutination of the strain RB5 in dilutions above 1 160

The highest agglutination with the serums from 6 healthy rabbits against strain RB5 was 1 40

respective serums are presented in table 3. The agglutination reactions obtained with joint fluids were surprisingly high. Control reactions performed with the typical arthritic strain AB13 yielded practically no agglutination.

#### DISCUSSION

In this study, agglutination reactions with the serums of patients with acute rheumatic fever have been performed against one organism, a non-hemolytic streptococcus, strain RB5, which was originally isolated from the blood of a patient with rheumatic fever. As previously stated, this organism contained antigenic properties in common with the majority of the strains of streptococci recovered in this laboratory from the blood and joints of patients with rheumatic fever. The agglutination tests have shown that the serums, as well as the joint fluids, of most patients with rheumatic fever contain agglutinins for strain RB5 in considerable amounts, while control serums possess little or no agglutinative power for this strain. The results of these agglutination reactions furnish additional evidence that non hemolytic streptococci are of etiologic importance in rheumatic fever.

The serums of 3 patients with chronic polyarthritis following rheumatic fever produced considerable agglutination of the rheumatic strain RB5, but little or no agglutination of the infectious arthritic strain AB13. These results suggest that the chronic polyarthritis which follows rheumatic fever is not etiologically identical with primary chronic infectious arthritis, and, furthermore, that the former disease is a direct sequel and chronic manifestation of rheumatic fever.

The agglutination reactions of 6 patients with subacute bacterial endocarditis were particularly interesting. The titers of agglutination against strain RB5 were unusually high, and afforded additional evidence of the relationship between rheumatic fever and subacute bacterial endocarditis. One is tempted to speculate as to just how close this relationship is. Are the two diseases identical, but expressions of different phases of a chronic streptococcal infection? Good arguments could be advanced for and against this theory, but such a discussion would be irrelevant in the present article.

In 4 patients with rheumatic fever studied at different stages of the disease, it was found that the agglutinin content of the serums rapidly

as well as during the active period. In each of the 4 cases the second study was made after the patient's temperature had been normal for about one month. The titers of agglutination during the febrile stage were 1 1280, 1 1280, 1 640, and 1 320, while during the convalescent stage they were 1 40, 1 160, 1 160, and 1 20, respectively. From these observations it appears that the agglutinins in the serums diminish rapidly following recovery from the disease.

#### *Stability of agglutinins in serums*

In order to determine the stability of the streptococcal agglutinins in the serums of patients with rheumatic fever, agglutination tests with strain RB5 were carried out at weekly intervals on the same

TABLE 3  
*Titers of agglutinations with joint fluids and corresponding serums from patients with rheumatic fever*

Patient	RB5* antigen		AB13† antigen	
	Joint fluid	Serum	Joint fluid	Serum
1	1 20	1 160	0	0
2	1 1,280	1 2,560	0	0
3	1 640	1 640	0	0
4	1 2,560	1 5,120	0	1 40
5	1 160	1 1,280	0	0

\* RB5 strain of streptococcus recovered from the blood of a patient with rheumatic fever.

† AB13 "typical strain" streptococcus recovered from the blood of a patient with chronic infectious arthritis.

serums. During this period the serums were kept in the refrigerator at almost freezing temperatures. In sharp contrast to the serums from patients with chronic infectious arthritis those from patients with acute rheumatic fever lost their power to agglutinate strain RB5 within 30 days after collection of the specimen.

#### *Streptococcal agglutinins in joint fluids*

Agglutination reactions with strain RB5 were carried out on the joint fluids of 5 patients with rheumatic fever. The technic for testing the joint fluids was similar in every respect to that employed for serums. The agglutinating titers of the joint fluids and of their

respective serums are presented in table 3. The agglutination reactions obtained with joint fluids were surprisingly high. Control reactions performed with the typical arthritic strain AB13 yielded practically no agglutination.

#### DISCUSSION

In this study, agglutination reactions with the serums of patients with acute rheumatic fever have been performed against one organism, a non hemolytic streptococcus, strain RB5, which was originally isolated from the blood of a patient with rheumatic fever. As previously stated, this organism contained antigenic properties in common with the majority of the strains of streptococci recovered in this laboratory from the blood and joints of patients with rheumatic fever. The agglutination tests have shown that the serums, as well as the joint fluids, of most patients with rheumatic fever contain agglutinins for strain RB5 in considerable amounts, while control serums possess little or no agglutinative power for this strain. The results of these agglutination reactions furnish additional evidence that non-hemolytic streptococci are of etiologic importance in rheumatic fever.

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The agglutination reactions of 6 patients with subacute bacterial endocarditis were particularly interesting. The titers of agglutination against strain RB5 were unusually high, and afforded additional evidence of the relationship between rheumatic fever and subacute bacterial endocarditis. One is tempted to speculate as to just how close this relationship is. Are the two diseases identical, but expressions of different phases of a chronic streptococcal infection? Good arguments could be advanced for and against this theory, but such a discussion would be irrelevant in the present article.

In 4 patients with rheumatic fever studied at different stages of the disease, it was found that the agglutinin content of the serums rapidly

diminished following recovery. In this respect, the agglutinins behave as in other infections such as typhoid fever and pneumonia, where they tend to disappear during or after convalescence.

#### CONCLUSIONS

1 Additional evidence is presented in support of the theory that streptococci of the non-hemolytic type are important etiologic agents in rheumatic fever. This evidence consists in the demonstration of streptococcal agglutinins in the serums of rheumatic fever patients.

2 Chronic progressive polyarthritis following rheumatic fever although presenting a clinical picture similar to primary chronic infectious arthritis gives evidence by agglutination reactions of being etiologically different.

3 Further evidence is presented of the etiologic relationship between rheumatic fever and subacute bacterial endocarditis.

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## STUDIES OF THE KIDNEY IN ACUTE INFECTION

### II OBSERVATIONS WITH THE UREA CLEARANCE TEST IN ACUTE RHEUMATIC INFECTION<sup>1</sup>

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In a previous study (1), we showed by means of the urine sediment count (2), that in acute rheumatic infection there is an abnormal increase in the excretion of formed elements in the urine for varying periods up to ten weeks following the acute stage of the disease. In that study, no instances of diffuse glomerulonephritis occurred.

Changes in renal function in the course of acute infections have been repeatedly investigated (3, 4, 5, 6, 7, 8, 9). These studies show a distinct lack of uniformity in the results obtained, and in their interpretation. This lack of agreement appeared to us to be in part due to the attempt to compare the results of different renal function tests. Those most frequently used were the blood urea nitrogen, phenolsulphonphthalein, and urea excreting power of the kidney. It is quite conceivable that these procedures not only test different functions of the kidney, but that they differ in their sensitivity to changes in renal function. Obviously then, they are not comparable. We felt that in order to obtain significant results in such a study, it would be necessary to select patients with the same infection of about the same degree of severity, and a single test sufficiently sensitive to indicate slight variations from normal. We decided upon the urea clearance test described by Möller, McIntosh and Van Slyke (10) shown to be highly sensitive as an index of the urea excreting activity of the kidney (13).

Acute rheumatic infection was selected as the first disease to be studied. Care was taken in the selection of patients, to obtain as far as

<sup>1</sup> The funds for this study were obtained from the Rheumatic Fever Fund Committee for Encouragement of Medical Research

possible, uncomplicated acute rheumatic infection. A few had acute fibrinous pericarditis and electrocardiographic evidence of acute myocardial involvement, but none showed evidence of congestive heart failure. In none of our group was there evident arteriosclerosis, hypertension or previous nephritis. Urine specimens for the clearance test were carefully collected by a group of specially trained nurses.<sup>2</sup> Blood and urine urea nitrogen determinations were made by the gasometric method described by Van Slyke (11).

The patients were all confined to bed during the period of investigation, and tests were invariably performed between nine A.M. and twelve noon (12). In all other respects the test was carried out exactly as prescribed by the authors (10). The factor measured by the clearance test is the volume of blood which a definite excretion of urine will clear of urea. When the urine volume is less than 2 cc per minute the "standard clearance" is employed, being the volume of blood which 1 cc of urine excreted in 1 minute suffices to clear of urea. When the urine volume is 2 cc or more per minute "the maximum clearance" is employed, being the volume of blood which one minute's excretion of urine suffices to clear of urea. In the average sized normal adult (surface area 1.73 square meters) the standard clearance varies from 41 cc to 65 cc of blood cleared of urea by 1 cc of urine in one minute, with a mean of 54 cc. For the same individual, the maximum clearance ranges from 64 cc to 99 cc of blood cleared of urea in one minute, with a mean of about 75 cc. Multiplying by the factors 1.85 or 1.33, standard or maximum clearance values respectively may be recorded on the basis of 100 per cent of normal renal function.

Table 1 indicates clearance values as determined thirty-four times in seventeen different individuals in whom there was no reason to suspect renal abnormality. The accepted normal range is from 75 per cent to 125 per cent of the average normal renal function, taken as 54 cc standard, and 75 cc maximum. Seven of our figures are below this range and one is above. These determinations, however, represent single readings. In each of the five individuals in whom three or more determinations were made, as well as in the entire group, the average results are well within the accepted normal range.

<sup>2</sup> The expense of this special nursing staff was met in part by the Crane Fund and in part by the Rheumatic Fever Fund of the Committee for Encouragement of Medical Research.

TABLE I  
Blood urea clearance in normal persons\*

Name	(V) Urine volume cc. per minute	$\sqrt{V_{cor}}$	(B) Blood urea nitrogen mgu. per 100 cc.	(U) Urine urea nitrogen mgu. per 100 cc.	Kidney function per cent of average normal
G	1 130	1 065	6 16	355 00	115 00
K	0 489	0 699	9 90	500 00	65 28
Mc	0 540	0 735	10 60	798 50	102 36
M	0 563	0 750	10 60	435 00	56 88
L	1 120	1 240	6 85	321 50	107 57
T	0 513	0 753	11 30	787 65	97 09
A	0 690	0 831	13 40	625 35	71 52
J	0 590	0 770	11 20	519 90	66 12
Tw	0 435	0 640	11 10	737 50	78 66
E	0 516	0 738	7 10	584 50	109 40
F	0 473	0 680	6 10	481 45	99 29
	0 324	0 640	13 17	1,167 60	100 90
D	7 100	7 640†	10 90	124 00	115 80†
	10 400	11 350†	8 30	48 40	90 04†
	7 450	6 780†	9 05	172 80	108 00†
C	1 140	1 050	14 60	760 50	102 20
	0 970	0 975	10 35	502 50	85 75
Di	0 623	0 790	8 10	459 70	83 80
	0 815	0 899	12 78	486 00	64 40
	0 669	0 790	17 00	1,021 50	88 30
	0 815	0 915	16 70	1,369 50	136 50
S	0 800	0 905	26 30	1,542 50	98 00
	0 580	0 765	20 00	1,371 50	97 60
	0 584	0 775	25 35	1,567 50	89 00
	0 625	0 895	21 80	1,557 00	107 00
	4 615	5 140†	15 40	258 30	96 90†
Sc	1 060	1 070	12 80	632 50	99 00
	1 000	1 030	10 83	746 00	104 50
	3 370	3 758†	9 85	252 00	125 50†
	0 975	1 935	15 70	842 50	91 00
	5 140	4 675†	15 90	239 50	86 10†
St	4 920	4 475†	11 56	183 75	86 75†
	3 700	3 200†	11 35	187 90	69 10†
	4 810	5 150†	9 57	95 12	66 13†
Average			12 74		93 28

\* Each result tabulated represents the average of two separate hourly determinations.

† Maximum clearance and  $V_{cor}$  instead of  $\sqrt{V_{cor}}$ .

‡ Volume corrected to surface area 1.73 sq. m. (14)

In table 2 are listed the results of the clearance test in sixteen patients with acute rheumatic infection. The test was performed seventy-five times at intervals during the active and convalescent periods.

Of the sixteen patients, eleven showed a distinct elevation of the clearance during the acute period of the disease, three showed normal values, and none showed a depression of the clearance value during the acute stage. The remaining two patients were first observed after the acute stage had passed.

Of the eleven patients who showed an abnormal elevation of the clearance during the acute stage, eight were followed into the convalescent period. In six of these there occurred a moderate to very striking depression of the clearance value during this period. Where it was possible to follow the patient sufficiently long after apparent recovery, the clearance was found to return to normal in from one to eighteen days. During the period of low clearance values, the blood urea nitrogen was normal, and the patients appeared well in every respect. That the urea clearance value may be very low in the presence of normal blood urea nitrogen and creatinine is an indication of its extreme sensitiveness as a test of renal function. It has been shown (13) that blood nonprotein nitrogen is invariably above normal only when the blood urea clearance indicates less than 20 per cent of normal renal function. In one instance, the patient insisted and did leave the hospital feeling perfectly well, although the clearance value showed only 32 per cent of normal kidney function.

Of the patients who showed normal clearance values during the acute stage of the disease, one was observed over a period of nine weeks, during which the clearance fell to 15 per cent of normal kidney function and was found to be normal thirteen days later. The intervals of thirteen and eighteen days represent maximum duration of the low clearance values, since, if more frequent observations had been made, it is quite likely that this period may have been found somewhat shorter.

In no instance was the clearance value found to be unusually low during the acute stage of the disease, and in no instance was it found unusually high except during this period.

The blood urea nitrogen exceeded our highest normal figure only in patient 14. We were unable to obtain further data on this patient.

TABLE 2  
*Urea clearance in acute rheumatic infection\**

Number	Name	Date	Blood	Urine urea	Urine	Per cent of	Temper
			urea nitrogen mgm per 100 cc.	urea nitrogen mgm per 100 cc.	volume corrected† cc. per minute	normal function per cent	
1	T	April 22, 1929 April 30, 1929	13.98	1,238.30	1,0270	165.93	101.8
			8.50	1,190.00	0.6090	204.96	99.6
Left hospital A O R.							
2	Du	April 23, 1929	25.50	1,152.70	0.9840	82.93	102.0
		May 7, 1929	10.90	853.50	0.8700	135.01	99.6
		June 4, 1929	10.30	486.50	2.2700	142.50†	99.6
		June 10, 1929	11.30	265.50	2.2990	71.54†	99.4
		June 21, 1929	11.50	316.00	1.3070	58.12	99.6
		August 9, 1929	9.99	275.20	2.4900	91.24†	97.6
3	N	April 24, 1929	17.48	1,397.70	0.9950	147.19	104.0
		May 3, 1929	11.60	1,273.00	0.8290	185.92	102.2
		May 8, 1929	12.00	1,392.50	0.5300	156.43	100.0
		May 17, 1929	13.80	611.50	0.8660	76.62	99.0
		July 19, 1929	17.34	760.00	0.5410	59.60	98.6
4	De	April 29, 1929	17.68	987.60	1.1550	111.62	103.6
		May 8, 1929	20.45	1,030.00	0.6760	75.48	100.4
5	B	May 1, 1929	24.70	1,231.50	0.8606	85.32	104.0
		May 20, 1929	18.80	917.00	0.6167	70.35	98.8
		June 14, 1929	10.70	633.50	0.5527	81.78	98.6
6	Tw	May 2, 1929	16.42	1,425.00	0.7879	141.76	102.2
Uncooperative							
7	C	May 4, 1929	10.92	1,425.00	0.7430	207.97	103.4
		May 13, 1929	10.32	1,495.50	0.7760	235.19	101.8
		May 20, 1929	20.35	1,282.50	0.8863	109.76	102.4
		May 24, 1929	15.40	999.00	1.1570	129.48	101.0
		May 28, 1929	15.20	822.50	0.8783	93.77	103.0
		June 4, 1929	11.90	482.00	0.7267	63.91	101.4
		June 11, 1929	18.08	380.00	0.2940	21.12	98.0
		June 21, 1929	10.70	151.00	1.0600	26.10	99.6
		June 25, 1929	10.62	332.00	0.8890	54.44	101.0
		June 28, 1929	9.93	389.45	0.7797	64.10	102.6
		July 10, 1929	5.90	139.10	0.6690	35.74	100.0
		July 23, 1929	3.35	136.96	0.6610	61.62	99.0

\* Each result tabulated represents the average of two separate hourly determinations.

† Maximum clearance.

‡ Volume corrected to surface area 1.73 sq m (14)

## KIDNEY STUDIES IN RHEUMATISM

TABLE 2—Continued

Number	Name	Date	Blood urea nitrogen	Urine urea nitrogen	Urine volume corrected†	Per cent of normal function	Temperature
Uncoöperative—Concluded							
8	M	May 16, 1929	16 90	787 50	0 4995	61 12	103 4
		May 22, 1929	18 39	1,119 50	0 8105	100 81	102 2
		June 3, 1929	12 69	382 00	0 4784	38 57	99 6
		June 13, 1929	12 45	306 00	0 2646	23 31	98 6
		June 24, 1929	9 63	317 00	1 1770	66 24	103 4
		July 1, 1929	4 48	210 30	0 5130	61 90	99 8
		July 11, 1929	11 20	112 32	0 7040	15 59	100 0
		July 24, 1929	9 87	462 90	0 7990	77 64	99 6
9	G	May 17, 1929	9 20	1,480 00	0 30195	164 08	104 8
		May 24, 1929	9 94	518 50	1 27200	135 12	103 6
		May 31, 1929	9 10	965 00	0 55440	141 74	104 0
		June 7, 1929	12 50	326 50	0 83320	41 90	102 0
		June 20, 1929	7 34	634 50	0 35255	6 60	100 0
		June 26, 1929	9 55	483 00	0 89210	28	99 2
10	A	April 3, 1930	22 55	1,564 50	0 76800	121 40	99 8
		April 10, 1930	26 20	501 00	1 25000	39 45	99 0
		April 11, 1930	16 22	466 00	2 94000	84 70†	99 6
11	C	January 24, 1930	14 12	1,516 00	0 56700	149 40	103 0
A O R							
12	Dl	October 29, 1929	4 40	711 00	1 07000	256 07	103 0
		December 6, 1929	7 54	122 40	4 40000	99 75†	102 0
		January 2, 1930	11 05	1,607 00	0 25700	34 67	99 8
		January 20, 1930	7 74	409 00	0 51500	70 60	97 8
		February 11, 1930	19 35	662 00	0 69500	52 25	99 0
		April 7, 1930	7 45	269 00	2 53000	105 25†	98 0
13	K.	November 14, 1929	19 10	1,453 00	1 25300	160 40	100 2
		December 4, 1929	13 48	990 55	0 93000	132 40	100 0
		December 30, 1929	14 70	775 00	0 70500	76 50	99 6
		January 23, 1930	11 10	576 75	0 75400	75 30	98 8
		January 31, 1930	17 00	315 50	1 34000	41 00	99 6
		February 7, 1930	14 68	499 50	1 68000	81 20	99 6
		February 17, 1930	12 50	407 00	2 00000	86 70†	99 0
		April 23, 1930	9 68	665 50	1 00000	125 40	98 0

TABLE 2—Concluded

Number	Name	Date	Blood urea nitrogen	Urine urea nitrogen	Urine volume corrected†	Per cent of normal function	Temperature
A O R.—Concluded							
14	P	February 21, 1930	43.30	1,666.00	0.70500	59.60	99.8
15	R	February 12, 1930	13.65	1,667.00	1.31000	255.00	100.0
		February 20, 1930	16.50	782.00	0.65000	78.80	99.8
		March 10, 1930	18.90	196.50	5.57500	75.90†	99.4
		March 20, 1930	16.67	772.50	1.72200	114.80	98.8
16	S	April 28, 1930	16.30	1,577.00	0.76200	152.30	100.0
		May 6, 1930	9.80	576.50	0.10000	41.15	99.8
		May 7, 1930	18.65	840.00	0.88100	79.92	100.0
		May 8, 1930	9.35	84.10	10.62000	106.25†	100.0
		May 13, 1930	16.65	539.50	1.08500	77.65	99.8
		May 15, 1930	9.80	274.50	2.57000	92.90†	99.6
		May 16, 1930	14.45	893.00	0.69000	95.70	99.6

The urea clearance test has proved itself to be a sensitive index of changes in renal function (urea excreting activity) (13). Under controlled conditions it has a fairly constant normal value (10). Any deviation from this must, we feel, be interpreted as deviation from normal renal function. The normal value is constant in spite of urea feedings (10). It is likewise uninfluenced by the feeding of high or low protein diets\*. It is evident from our figures that both high and low clearance values are independent of both blood urea nitrogen and urine volumes. The standard urea clearance values vary directly with the urea concentrating power of the kidney  $\left(\frac{U}{B}\right)$ . If we accept the empirical clearance values determined in normal persons as an indication of average normal renal function, our results in rheumatic infection must be interpreted as indicating a period of renal hyperfunction during the active febrile course of the disease, and renal hypofunction during the afebrile convalescent period. In this sense, renal hyperfunction may be conceived as a compensatory effort to remove from

\* Unpublished data (author).

the blood and tissues, as rapidly as possible, the urea accumulating as a result of heightened metabolism. Whether the mechanism involved is increased glomerular blood flow or increased glomerular activity, enhancing more rapid urea filtration or more rapid tubular secretion of urea, is not possible to say at this time. In either event, the kidney is responding to some stimulus by a degree of functional activity exceeding the observed normal. Similarly, in the immediate postfebrile period, the kidney loses in varying extent, its capacity for excreting urea. It may be that diminished glomerular flow accounts for this subnormal phase. If, indeed, a structural basis is to be predicated, it may be that the renal functional change noted, is the result of the diffuse cloudy swelling which accompanies febrile states. Whatever the renal damage, it is only temporary, and as our tables show, capable of complete functional restitution. Since the urea clearance test was employed consistently throughout this study, we have been investigating only one particular function of the kidney, namely, its urea excreting activity. The fact that other renal function tests may not show the same results as we have observed would merely mean that either they did not test kidney function with the same degree of sensitivity as the urea clearance, or that they test some other particular function of the kidney. We feel that the extreme sensitivity of the urea clearance test renders it invaluable in a study of this nature, where changes in renal function are apt to be slight.

#### CONCLUSIONS

Values for the standard and maximum urea clearance tests have been determined in normal persons.

It has been shown that in the acute febrile stage of rheumatic infection, clearance values are usually higher than the highest observed normal, and during the afebrile convalescent period, the clearance values are usually lower than the lowest observed normal.

These findings have been interpreted as indicating a state of renal hyperfunction during the acute stage as a response to the demand of increased protein catabolism, and a state of renal hypofunction probably as a result of toxic injury to the kidney parenchyma.

In all patients followed, complete restoration of renal function occurred within about two weeks.

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## STUDIES OF THE KIDNEY IN ACUTE INFECTION

### III OBSERVATIONS WITH THE URINE SEDIMENT COUNT (ADDIS) AND THE UREA CLEARANCE TEST IN LOBAR PNEUMONIA<sup>1</sup>

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McIntosh and Reimann (1) reviewed some previous studies on kidney function in pneumonia. They found that of six investigators, three reported impairment of renal function (2, 3, 4), two reported the absence of impairment (5, 6), and one (7) emphasized the occurrence of renal hyperfunction during the course of lobar pneumonia. It seemed to them that a further series of observations was necessary to throw some light, if possible, on the cause for such discordant results. The tests employed were the phenolsulphonphthalein test and the urea concentration index (8). Thirteen patients were studied. They reported renal hyperfunction, by one or the other of the tests employed, in nine patients and moderate depression of renal function in four. In no instance was there encountered serious impairment of renal function.

In a previous study (9), we showed by means of the urine sediment count, that during the course of acute rheumatic infection, there is an abnormal increase in the excretion of formed elements in the urine, persisting for some weeks after recovery, with a final return to normal. In another study (10) we showed by means of the urea clearance test, that during the acute phase of rheumatic infection there occurred a period of renal hyperfunction, followed, in the convalescent phase by a period of renal hypofunction. The latter functional state persisted for a varying number of days and in every instance where the patient was observed over a sufficient period of time, it was followed by com-

<sup>1</sup> Funds for this study were obtained from the Rheumatic Fever Fund of the Committee for Encouragement of Medical Research.

## KIDNEY STUDIES IN PNEUMONIA

TABLE I  
*Urine sediment count in normal persons*

Number	pH	Specific gravity	Urine volume per 12 hours	Protein per 12 hours*	Red cells per 12 hours	White and epithelial cells per 12 hours	Casts per 12 hours†
			cc	mgm	millions	millions	thousands
1	5.0	1.030	369	5.3	0	0.220	9.2
2	5.0	1.040	259	18.0	0	0.064	0
3	5.0	1.031	473	10.2	0	0.118	0
4	5.5	1.023	355	12.8	0	0.117	0
5	5.0	1.044	208	8.9	0	0.062	0
6	5.0	1.020	616	22.1	0	0.077	0
7	5.5	1.010	776	8.4	0	0.580	0
8	5.0	1.027	228	32.8	0	0.300	3.8
9	5.8	1.027	408	57.7	0	0.730	0
10	5.5	1.027	269	23.1	0	0.670	2.2
11	6.7	1.032	186	26.8	0	0.600	4.1
12	5.6	1.028	337	24.8	0	0.400	0
13	5.0	1.036	147		0.009	0.082	0
14	{	5.8	1.030	259	18.6	0.010	0.790
		5.7	1.028	234	16.8	0.260	1.100
		5.5	1.028	285	42.0	0.010	0.810
15		5.0	1.040	158	5.6	0.013	0.115
16		5.5	1.018	145	10.4	0.020	0.290
17		5.0	1.035	170	18.3	0.020	0.360
18		5.0	1.028	80	5.8	0.022	0.520
19		5.5	1.032	335	21.7	0.027	1.360
20		5.5	1.026	343	17.2	0.028	0.056
21		5.0	1.038	274	60.2	0.034	0.180
22		6.5	1.027	360	25.9	0.040	0.470
23	{	5.5	1.022	344	49.5	0.050	0.270
		5.0	1.027	186	26.6	0.090	0.720
		5.5	1.036	226	26.2	0.060	0.190
24		5.8	1.029	239	29.3	0.070	0.370
25		5.7	1.020	363	52.2	0.070	0.700
26		5.0	1.032	287	15.4	0.071	0.262
27		6.5	1.024	387	13.9	0.072	0.097
28		5.0	1.034	137	2.9	0.076	0.340
29		5.3	1.034	220		0.088	0.638
30		5.0	1.034	120	7.8	0.127	3.400
31		5.5	1.037	187	26.9	0.130	0.280
32		5.5	1.030	237	34.2	0.140	0.630
33		5.0	1.036	414	17.9	0.145	0.028
34		5.5	1.042	210	9.0	0.157	0.131
35							0
36							0

\* Centrifuge method (see reference 15)

† All were of the hyaln variety

TABLE 1—*Concluded*

Number	pH	Specific gravity	Urine volume per 12 hours	Protein per 12 hours	Red cells per 12 hours	White and epithelial cells per 12 hours	Casts per 12 hours†
			cc.	mgm.	millions	millions	thousands
37	6.5	1.026	385	22.1	0.159	0.192	0
38	5.5	1.027	205	10.2	0.205	0	
39	6.0	1.031	312	33.7	0.160	0.810	0
39	5.0	1.030	348	30.1	0.200	0.870	3.8
	5.8	1.031	256	28.7	0.210	0.320	0
41	5.5	1.033	384	46.0	0.300	0.830	6.4
42	5.5	1.028	302	6.5	0.390	1.040	0
43	5.0	1.030	293	42.2	0.560	2.430	0
44	5.0	1.033	218	22.3	0.650	1.270	4.8
45	5.5	1.038	355	15.3	0.931	0.047	3.9
	5.0	1.043	280	10.0	1.530	0.024	0
Average				21.8	0.146	0.540	1.3

plete restitution of normal renal function. Since there was no reason to believe that such findings should be peculiar to acute rheumatic infection alone, we decided to repeat the same observations in a group of patients with lobar pneumonia.

Adult patients were selected from a series under investigation by the pneumonia service. No patients were selected who presented signs or gave a previous history of congestive heart failure, nephritis, or hypertension. Patients who developed reaction from pneumococcus serum were likewise excluded. The urine sediment counts were performed according to the technique described by Addis (11). The urea clearance test was performed according to the technique described by Möller, McIntosh and Van Slyke (12), and McKay (13). Blood and urine urea nitrogen determinations were made by the gasometric method of Van Slyke (14). Both urine sediment counts and blood urea clearances were made at intervals during the acute stage of the disease and during convalescence.

Table 1 indicates the results of 49 urine sediment counts performed on 45 medical students in whom there was no reason to suspect abnormality in the kidneys. The urinary protein was determined by the method described by Shevky and Stafford (15). We felt it was reasonable to accept as the upper limit of normal excretion for a

## KIDNEY STUDIES IN PNEUMONIA

TABLE 2  
Quantitative study of the 12 hour urine sediment in lobar pneumonia

Name	Number	Date	Urine volume per 12 hours	pH	Specific gravity	Protein Per 12 Hours	Red blood cells per 12 hours	White and epithelial cells per 12 hours	Casts per 12 hours	Temperature	Date of termination
			cc			mgm	millions'	millions	Thousands	°F	
A	1	March 26, 1930	118	0.5	0.1010	200	00	0.06	0.70	13.0	March 27, 1930
		March 29, 1930	153	0.5	0.1016	90	00	0.20	1.48	0.0	102.6
Ba	2	April 9, 1930	143	0.5	0.1010	2,162	00	13.50	5.24	1,120.0	Onset of diffuse glomerulonephritis April 9, 1930
Bb	3	April 12, 1930	251	0.5	0.1013	2,349	00	37.37	7.90	2,649.0	99.2
Bc	4	May 15, 1930	1,520	0.5	0.1007	4,529	00	11.160	0.00	0.15	0.0
Bd	5	January 25, 1930	636	0.5	0.1020	230	40	46.43	2.45	44.2	January 26, 1930
Bf	6	February 8, 1930	680	0.5	0.1008	97	00	0.60	1.11	28.3	99.6
Bg	7	October 25, 1929	510	0.5	0.1020	826	00	0.13	1.45	7.1	Died October 26, 1929
Bh	8	March 13, 1930	400	0.5	0.1026	2,000	00	0.05	2.45	0.0	Died March 16, 1930
Bj	9	March 25, 1930	844	0.5	0.1013	90	00	1.27	6.00	3,446.0	Died March 26, 1930
Bk	10	January 21, 1930	910	0.5	0.1022	255	00	0.25	1.50	0.0	102.2
Bm	11	January 27, 1930	380	0.5	0.1022	164	20	0.19	6.68	460.0	January 29, 1930
Bn	12	December 11, 1929	184	0.5	0.1034	39	70	0.83	1.40	92.0	December 10, 1929
Bp	13	February 5, 1930	392	0.5	0.1018	197	60	0.00	1.47	147.0	February 6, 1930
Bq	14	December 31, 1929	705	0.6	0.1018	110	00	0.38	2.77	2,270.0	December 30, 1929
Bs	15	December 18, 1929	404	0.5	0.1022	20	00	0.00	0.00	0.0	Died December 21, 1929
Bt	16	April 12, 1930	357	0.5	0.1018	899	50	0.54	1.88	79.5	102.0
Bv	17	April 24, 1930	286	0.5	0.1018	113	00	0.93	6.00	63.0	Empyema May 7, 1930
Bw	18	December 20, 1929	576	0.5	0.1018	36	00	0.59	2.66	30.0	102.6
Bx	19	December 30, 1929	430	0.5	0.1030	154	00	9.90	418.00	0.0	99.6
Bz	20	January 4, 1930	340	0.5	0.1032	73	40	0.09	2.34	4.7	100.0
Baa	21	January 8, 1930	265	0.5	0.1024	133	80	6.63	400.00	0.0	100.0
Bab	22	February 7, 1930	760	0.5	0.1008	300	90	1.71	0.09	0.0	100.0
Bac	23										February 7, 1930

F	14	January 11, 1930	460	0	1	026	62	20	0	00	1	60	0	0	100	6	January 11, 1930		
Ga	15	March 4, 1930	484	05	5	1	0111	036	80	0	36	1	50	0	0	101	0	March 6, 1930	
Gb	16	April 4, 1930	702	05	0	1	0228	027	00	2	63	9	48	20	0	105	0	Died April 6, 1930	
Gc	17	March 7, 1930	730	05	0	1	0181	576	00	30	84	4	01	9	733	0	Died March 25, 1930		
Gd	18	April 1, 1930	327	05	0	1	020	150	00	0	08	0	57	45	4	103	8	April 3, 1930	
Ge	19	February 7, 1930	1,424	05	0	1	010	153	00	0	00	6	10	0	0	105	0	March 1, 1930	
Gf	20	March 19, 1930	511	05	5	1	022	490	00	8	34	94	2	99	0	0	105	0	March 19, 1930
Hb	21	March 24, 1930	710	05	0	1	025	50	00	0	35	2	10	9	7	99	2		
Ka	22	January 11, 1930	570	05	0	1	026	102	00	0	14	2	00	31	6	101	6	January 11, 1930	
Kb	23	February 22, 1930	367	05	0	1	015	85	50	0	92	6	42	2	230	0	103	8	February 24, 1930
Kc	24	April 25, 1930	480	05	0	1	0101	109	00	0	12	2	52	100	0	104	0	April 29, 1930	
Kd	25	February 3, 1930	186	05	0	1	032	133	90	0	14	0	74	6	8	103	2	February 5, 1930	
La	26	April 23, 1930	125	05	5	1	011	32	00	0	00	0	09	0	0	103	6	May 9, 1930	
Ma	27	January 21, 1930	227	05	0	1	030	326	88	0	00	2	09	6	3	102	2		
Mb	28	October 24, 1929	282	05	5	1	017	20	39	0	07	0	70	0	0	103	6	Died November 12, 1929	
Mc	29	December 31, 1929	303	05	5	1	020	359	90	0	37	1	89	993	2	102	8	Died January 1, 1930	
Md	30	January 25, 1930	98	05	0	1	020	21	20	0	01	0	09	0	0	101	0	January 28, 1930	
Me	31	February 7, 1930	327	05	0	1	018	109	00	0	04	0	02	45	1	99	6		
Ob	32	January 4, 1930	1,168	05	5	1	014	336	40	0	58	0	58	0	0	102	2	January 5, 1930	
Pa	33	January 8, 1930	780	05	0	1	015	168	00	0	09	1	46	0	0	100	6		
Sa	34	May 9, 1930	2,592	05	5	1	001	170	00	0	77	28	42	0	0	99	8	April 16, 1930	
Sb	35	May 14, 1930	1,028	05	0	1	003	140	00	0	51	9	25	0	0	100	2	Onset of diffuse glomerulonephritis May 3, 1930	
Ob	36	May 21, 1930	941	05	0	1	007	135	00	0	80	1	56	0	0	99	6		
Os	37	February 26, 1930	707	05	0	1	014	267	12	0	09	2	74	3,731	0	103	8		
Ob	38	January 29, 1930	250	05	5	1	0151	980	00	3	70	2	77	0	0	102	0		
P*	39	October 22, 1929	97	05	0	1	028	1	40	0	06	0	23	5	4	98	8		
Sa	40	March 24, 1930	463	05	0	1	014	572	24	1	16	0	00	0	0	105	0	Died March 24, 1930	
Sb	41	April 22, 1930	289	05	5	1	0181	1,500	00	0	96	11	16	6	036	8	104	4	Died April 23, 1930

TABLE 2—*Concluded*

Name	Number	Date	Urine volume per 12 hours	pH	Specific gravity	Protein per 12 hours	Red blood cells per 12 hours	White and epithelial cells per 12 hours	Casts per 12 hours	Temperature °F	Date of termination
			cc.			mgm	millions	millions	thousands		
Sc	37	March 18, 1930	620	0	1.019	691	90	1.70	27.59	0 0	March 22, 1930
Sd		March 25, 1930	1,064	0.5	1.010	170	00	6.38	291.54	0 0	99 8
Sd	38	October 26, 1929	410	0	1.028	30	50	0.05	0.71	5 7	Died October 30, 1929
Ta	39	April 10, 1930	436	0.5	1.024	25	00	0.00	1.80	57 6	Died April 13, 1930
Us	40	March 12, 1930	788	0.5	1.001	195	00	0.09	2.07	0 0	February 6, 1930
Va	41	May 20, 1930	684	0	1.018	2,462	00	0.00	0.68	38 0	May 8, 1930
	Wb	May 15, 1929	598	0	1.026	14	68	1.04	2 23	1,605 9	99 2
		May 20, 1929	415	0.5	1.026	180	00	0.78	1.38	553 0	99 0
	Wa	May 23, 1929	590	0	1.028	53	00	0.49	0.79	190 0	98 6
		May 31, 1929	583	0	1.023	37	80	0.17	1.92	372 0	98 6
	Wb	February 14, 1930	650	0	1.011	193	70	1.54	27.44	0 0	February 18, 1930
		February 22, 1930	113	0.5	1.018	24	40	0.56	17.80	0 0	99 0
	Za	February 18, 1930	388	0	1.022	83	80	0.63	1.55	882 6	104 6
		February 24, 1930	433	0.5	1.020	124	70	1.95	1.41	0 0	February 24, 1930
	Za	March 3, 1930	427	0	1.008	122	98	20.33	29.46	0 0	99 6

period of 12 hours, 500,000 red blood cells, 1,000,000 white blood cells, and epithelial cells, 5,000 casts, and 30 mgm of protein (16)

Table 1 in the preceeding paper, indicates the results of 34 blood urea clearance determinations in 17 patients and medical students in whom there was no past or present evidence of renal disease. The normal range varies from 75 per cent to 125 per cent of normal renal function.<sup>2</sup>

Table 2 shows the results of the urine sediment counts in 44 patients with lobar pneumonia. By comparison with table 1, it is evident that at some period during the precritical stage of lobar pneumonia, 38 of 44 patients showed an abnormal excretion of protein and one or more of the formed elements. There were 40 sediment counts made when the temperature was 101°F or higher. Of these, protein was excreted in greater amount than 30 mgm per 12 hours 34 times. Casts were excreted in greater number than 5,000 per 12 hours 26 times. White blood cells and epithelial cells were excreted in greater number than 1,000,000 per 12 hours 29 times. However red blood cells were excreted above the normal rate of 500,000 per 12 hours only 16 times. The occurrence of microscopic hematuria in the course of lobar pneumonia appears to be of greater significance than the occurrence of abnormal excretion in the urine of the other formed elements or protein (16). The extent of the microscopic hematuria appears to carry no significance as far as its interpretation goes. Of more importance is the time of occurrence of hematuria and its subsequent behavior. This fact is demonstrated by two of our patients. Patient 1 had a fairly normal urine sediment before and immediately after the crisis. However, microscopic hematuria made its first appearance 12 days after the crisis and on repeated tests this progressed to frank bloody urine. The almost concomitant occurrence of hypertension and generalized edema with progressive renal functional failure indicated the development of acute diffuse glomerulonephritis. Part of the clinical course in this patient is shown in chart 1. The same complication was observed in patient 31 with the exception that the hematuria was entirely microscopic, practically negligible in degree, and cleared up more rapidly. The clinical course in this patient is

<sup>2</sup> For further details of the blood urea clearance test see reference (12).

shown in chart 2 Whether the pneumococcus or a secondarily invading streptococcus was responsible for the nephritis in these two patients is not possible to say with certainty

Of entirely different significance is the hematuria which occurred in patients 2, 20, 30, 42, and 43 In these patients microscopic hematuria occurred before or about the time of the crisis, disappearing completely, promptly after the crisis With the onset of microscopic

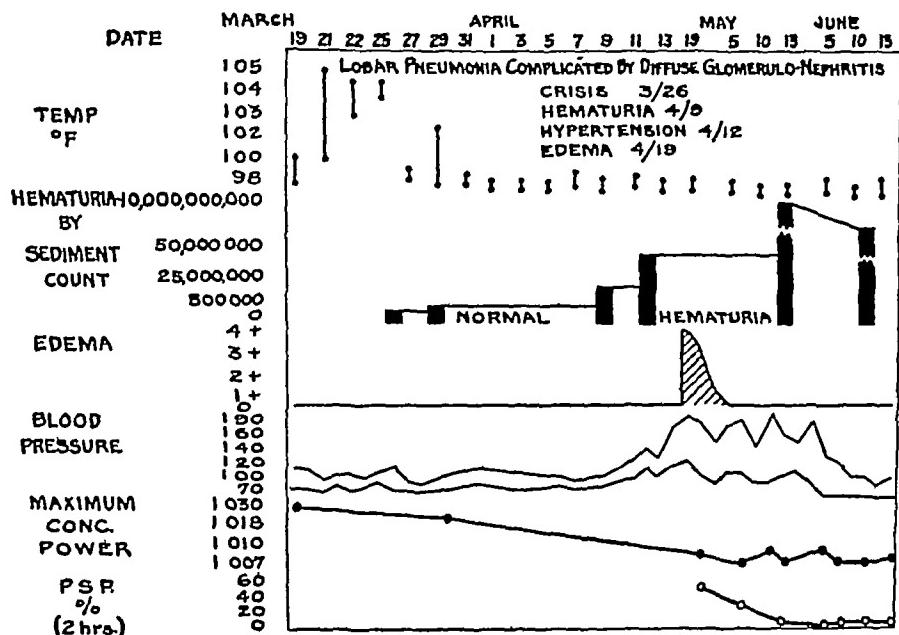


CHART 1 Patient A, Number 1 White, male, aged 39 Hematuria was first noted 14 days after the crisis Then followed a rise in blood pressure, generalized edema including the face and impairment of renal function Hematuria and impaired renal function were present at a subsequent examination, 2 months after discharge

hematuria during the acute phase of the disease, its prompt disappearance and the absence of systemic manifestation, hypertension, and edema, these cases must be interpreted as instances of focal glomerulitis and are of no prognostic significance In patient 44 the occurrence of hematuria, for the first time, in convalescence is highly suggestive of the onset of diffuse glomerulonephritis, but the absence of hypertension or edema and the lack of renal function study, makes it

impossible to be certain of any more than a focal glomerular lesion. The appearance or disappearance of microscopic hematuria apparently bears no relationship to the excretion rate of the other formed elements or protein. These appear to be of different pathogenesis as well as of different significance as far as our observations go. Apparently a more severe renal lesion is necessary to permit the excretion of red

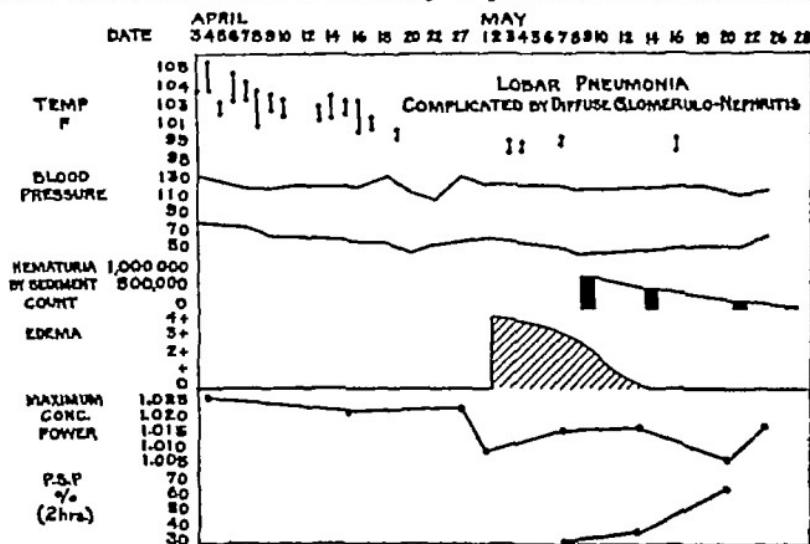


CHART 2 Patient Me, Number 31. While male, aged 31. Generalized edema involving the face was first noted 13 days after the crisis. Distinct hematuria was not present one week after the onset. The blood pressure did not rise, but renal function was temporarily impaired. Patient left the hospital apparently well.

blood cells. This fact had been previously observed in studying the urine sediment in congestive heart failure (16).

In table 3 are shown the results of the blood urea clearance test during the acute and convalescent stages of lobar pneumonia. Our results confirm the findings of McIntosh and Reimann (1). Of 13 patients studied, 9 showed a period of renal hyperfunction (increased urea excreting activity) during the acute stage, 2 showed a period of renal hypofunction (decreased urea excreting activity) during the acute stage, and 2 showed no change from normal function. Of the 9

## KIDNEY STUDIES IN PNEUMONIA

TABLE 3  
Blood urea clearance test in lobar pneumonia\*

Name	Number	Date	(B) Blood urea nitrogen	(U) Urine urea nitrogen	$\frac{\text{msec}}{100 \text{ cc}}$	(V cor.) Urine volume corrected	Kidney func- tion per cent of average normal	Temperature per cent	°F	Result
Aa	1	March 25, 1930	24.8	1,309.0	1.310	112.0	103.0	Crisis March 27	103.0	Crisis March 27
		April 8, 1930	31.95	1,096.0	0.909	60.7	99.2			
		April 16, 1930	31.5	591.0	1.245	39.8	99.6			
		April 17, 1930	15.5	480.0	1.425	68.4	98.4			
		May 21, 1930	12.8	474.0	1.825	9.3	99.0			
		October 24, 1929	12.85	427.5	3.410	139.0†	102.8			
		March 14, 1930	25.9	933.5	2.785	133.8‡	104.2			
		March 15, 1930	40.2	604.5	0.990	27.7	103.6			
Bb	2	April 8, 1930	19.2	1,415.0	1.770	181.0	103.8	Died October 26, 1930	102.8	Died October 26, 1930
		April 15, 1930	5.85	1,149.0	0.675	299.0	101.8			
		April 22, 1930	11.17	1,228.0	0.774	178.5	103.0			
		April 29, 1930	13.28	1,339.0	0.515	134.9	103.0			
		May 5, 1930	13.6	1,136.0	0.475	106.8	102.6			
		April 2, 1930	14.2	1,140.0	1.175	162.0	102.0			
		April 2, 1930	10.35	1,547.0	0.860	256.7	103.4			
		April 7, 1930	7.06	630.5	1.242	186.5	99.0			
Cc	3	April 14, 1930	15.45	1,716.0	0.423	133.0	99.0	Empyema Died April 3 Crisis April 3	102.0	Died April 3 Crisis April 3
		April 21, 1930	13.65	660.0	0.798	79.5	99.0			
		April 25, 1930	10.42	795.0	0.4975	99.6	98.6			
		May 1, 1930	15.95	521.5	1.385	71.1	98.6			
		March 17, 1930	20.8	1,314.0	0.935	113.1	100.0			
		October 23, 1929	8.4	995.0	0.780	192.5	103.0			
Gd	6							Crisis March 19	103.0	Died November 12
Ha	7							Crisis March 19	103.0	Died November 12
Ma	8							Crisis March 19	103.0	Died November 12

		March 19, 1930	22.85	1,627.0	0.935	128.1	105	Crisis March 22
Sc	9	March 24, 1930	7.7	1,080.0	1,200	288.0	100	
		March 27, 1930	13.4	324.0	3,695	119.0†	99	
		April 1, 1930	12.9	93.0	6,430	61.6‡	98.8	
		April 4, 1930	12.2	90.1	7,470	73.5‡	100.0	
Ta	10	November 12, 1929	8.57	1,339.0	1,520	356.5	104.0	Crisis November 13
Ua	11	March 5, 1930	22.7	740.0	1,250	67.6	103.0	Crisis February 6
		March 18, 1930	13.55	185.2	1,088	25.4	99.6	
		May 22, 1930	8.3	129.0	7,150	147.8‡	103.2	
Va	12	May 28, 1930	7.97	595.0	0,893	136.5	98.8	May 27
		June 2, 1930	6.82	194.8	1,850	72.0	98.6	
		June 3, 1930	15.7	418.0	0,877	46.1	98.6	
		June 4, 1930	14.5	432.0	0,770	48.2	98.6	
Wa	13	May 9, 1929	80.5	638.0	0,323	8.35	99.8	May 18, 1929
		May 13, 1929	17.2	1,245.0	0,530	70.5	99.0	
		May 16, 1929	14.9	892.0	0,586	83.0	99.4	

\* Each clearance value represents the average of two separate hourly determinations.

† Corrected for ideal body surface area (1.73 square meters).

‡ Maximum blood urea clearance.

patients who showed hyperfunction, 3 showed a depression of renal function following the crisis. With 4 exceptions in the entire group, all remained normal or returned to normal after a period of depressed renal function. Of these four exceptions, in only one (patient 1) did we have sufficient correlated data to establish the diagnosis of diffuse glomerulonephritis. This same likelihood is quite possible in the other three instances (patients 3, 11, 12). Return of normal function occurred in from 3 to 7 days. Patient 13 is of interest since during the acute stage of the disease he developed a blood urea clearance of 8.35 per cent of normal renal function and a blood urea nitrogen of 80.5 mgm per 100 cc of blood. Seven days later, the results of both tests were normal and he made an uneventful recovery.

#### CONCLUSIONS

1. Observations with the urine sediment count, have been made in 44 adults with lobar pneumonia. Deviations from normal sediment findings have been presented and discussed. The occurrence of microscopic hematuria appeared to be of greater prognostic significance than the appearance of the other formed elements or protein in the urine.

2. Observations with the blood urea clearance test were made in 13 patients with lobar pneumonia. Renal hyperfunction (increased urea excreting activity) was the rule during the acute stage of the disease. Slight to marked depression of renal function (decreased urea excreting activity) occurred during both the acute and convalescent stages.

3. In two patients of this series there was sufficient correlated clinical data, to be certain of a complicating acute diffuse glomerulonephritis.

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## BODY BUILD IN INFANTS

### I THE TECHNIQUE OF MEASURING THE EXTERNAL DIMENSIONS OF THE BODY IN INFANTS

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#### ABSTRACT OF PAPERS I, II AND III

In these three papers the body build of infants during health and disease is described quantitatively. This is done by comparison of ratios of various external dimensions to the total body length. In the first paper the technique for measuring the external dimensions of the body in infants is described and various sources of error discussed.

In the second paper general empirical formulae for the relationship between various external dimensions and total body length in healthy infants are developed. By comparing two groups of infants from different social environments, it is shown that environment may influence body build.

In the third paper the results obtained in the second paper for healthy infants are used for comparison with sick infants. Various differences in the body build of infants with acute intestinal intoxication, tetany and eczema are described.

This study was designed as an investigation of the relationship between constitution and disease in infants. By constitution, as referred to living things, we mean the sum total of structural and functional properties which result from the reaction of the genetic make up to its environment. This definition is similar to that of Draper (1) and differs from that of J. Bauer (2) who looks upon constitution as the sum of the characteristics and attributes, transmitted through the germ plasm, and already present at the moment of conception.

Draper defines human constitution as "that aggregate of hereditary characters, influenced more or less by environment, which determines the individual's reaction, successful or unsuccessful, to the stress of environment."

Bauer defines constitution as "aus der Summe der durch das Keumplasma übertragenen, also schon im Momente der Befruchtung anlagenmassig gegebenen Merkmale und Eigenschaften" "Den gesamten Komplex von Erbanlagen, der die Zugehörigkeit des werdenden Individuums zu seiner Spezies, Rasse und Familie sowie zu seinem Sexus bestimmt, und bei der ungeheuren Mannigfaltigkeit und praktisch absoluten Originalität der Erbanlagenmischung den Grundstock der persönlichen Individualität ausmacht, bezeichnen wir als Konstitution"

The difficulty of dissociating heredity from the environment, as implied in the definition by Bauer, is evident from numerous experiments. Morgan (3) studied a mutant race of *Drosophila* called vestigial, because only vestiges of the wings are present. When the larvae were bred at the proper temperature, the vestigial forms appeared regularly. When, however, the larvae were bred at a temperature of about 31°C, the rudimentary wings were much longer and in many instances as long as the wings of the wild type. E. Baur (4) was able to produce different colored primroses by varying the temperature at which the plants were grown. When red primrose (*Primula Sinensis Rubra*), which ordinarily, at a temperature of 15 to 20°C, produces red flowers, is transplanted to a hothouse at a temperature of 30 to 35°C, the flowers which appear are white. When the plants are returned to a cooler place, they again produce red flowers. Similarly, Dorfmeister (5) in 1879 was able to produce butterflies with different colored wings by varying the temperature at which the larvae were grown. These experiments show clearly how environmental factors may influence the somatic expression of the hereditary material.

For convenience of study, constitution has been divided into various constituents, such as sex, race, body build, skin color, etc. In a recent paper the influence of the sex factor in infant mortality was discussed (6). That race is a factor in the greater prevalence of tetany among Negro infants was shown in studies on dosage of ultraviolet radiant energy (7).

This study is concerned with the relationship between body build and disease in infants. By body build we mean the external form of the body as it is determined by the skeletal and cartilaginous parts

The present paper is devoted to a description of the technique used in this study.

The instruments employed were the anthropometer described by Martin, the small sliding compass (Gleitzirkel), the small spreading calipers (Tasterzirkel), a steel tape and a measuring board similar to that described by Schultz (8).

All instruments were calibrated to 1 mm and all measurements were made to 1 mm. All unilateral measurements were made on the right side. In general, the dimensions measured and the technique employed were those described by Hrdlicka (9).

#### DESCRIPTION OF MEASUREMENTS

1 *Total body length* From the vertex to the sole with the foot at right angles to the body, taken parallel to the long axis of the body. The measuring board was found to be more accurate than the anthropometer for this measurement and the anthropometer was therefore discarded in the later work.

2 *Body weight* Measured in the usual way.

3 *Sitting height* From the vertex to the most caudal point over the ischial tuberosities, parallel to the long axis of the body.

4 *Circumference of head (occipitofrontal)* The largest circumference of the head.

5 *Cephalic length* From the most prominent part of the occiput to the glabella in the mid sagittal plane.

6 *Cephalic breadth (biparietal diameter)* The greatest width of the head between the parietal eminences, perpendicular to the mid sagittal plane.

7 *Diameter of face (bimalar)* The horizontal distance between the 2 malar prominences.

8 *Bigonal diameter* The horizontal distance between the most distant points of the angles of the jaw, perpendicular to the mid sagittal plane.

9 *Upper facial length* From the nasion to the prostion in the mid-sagittal plane.

10 *Height of lower jaw* From the infradentale to the menton in the mid sagittal plane.

11 *Height of nose* From the nasion to the nasal septum where it joins the upper lip.

12 *Breadth of nose* The greatest breadth between the nasal wings without exerting pressure Care should be taken that the child is quiet when this measurement is being taken

13 *Inter-inner canthus breadth* The horizontal distance between the inner angles of the eyes

14 *Length of palpebral fissure* From the internal to the external angle of the eye

15 *Length of ear (maximum)* From the highest point on the border of the helix to the lowermost point on the lobule, perpendicular to the long axis of the ear

16 *Breadth of ear* Distance between 2 lines, parallel to the long axis of the ear, one of these lines being tangent to the anterior, the other to the posterior border of the helix

17 *Circumference of the thorax at nipples* Taken half way between inspiration and expiration The respiratory excursion of the chest of a quiet infant is small

18 *Biacromial diameter* Straight distance between the most lateral points of the acromial eminences, taken from behind with the child seated, the arms close to the thorax

19 *Bicristal diameter* Straight distance between the most lateral points of the iliac crests, perpendicular to the mid-sagittal plane

20 *Length of humerus* From the most lateral point of the acromial eminence to the most proximal point on the lateral side of the capitulum of the radius in a plane parallel to the long axis of the humerus This measurement was made with the arm close to the thorax and the forearm extended

21 *Length of radius* From the most proximal point on the lateral side of the capitulum of the radius to the tip of the styloid process of the radius, parallel to the long axis of the radius

22 *Length of hand* From the tip of the styloid process of the radius to the tip of the middle finger taken in a plane parallel to the long axis of the forearm

23 *Length of palm* From the tip of the styloid process of the radius to the fold between the middle finger and the palm, in a plane parallel to the long axis of the forearm

24 *Breadth of palm* With the palm facing forward, from the most lateral point overlying the capitulum of the 2nd metacarpal bone, to

the most medial point overlying the capitulum of the 5th metacarpal bone in a line perpendicular to the long axis of the palm

25 *Length of middle finger* From the metacarpophalangeal joint of the middle finger to the tip

26 *Length of thigh* From the antero-superior spine of the ilium to the lower margin of the internal condyle of the femur in a plane parallel to the long axis of the body

27 *Length of tibia* From the lower margin of the internal condyle of the femur to the tip of the internal malleolus of the tibia with the leg extended at the knee

28 *Height of foot* This was obtained by subtracting from the knee-sole measurement the length of tibia. Knee sole was measured from the lower margin of the internal condyle of the femur to the sole, with the foot at right angles to the leg, in a plane parallel to the long axis of the leg

29 *Length of foot* From the point on the heel over the tuber calcanei which projects furthest back to the tip of the great toe with the foot held at right angles to the leg, in a line parallel to the internal margin of the foot.

30 *Breadth of foot* From the most lateral point overlying the capitulum of the fifth metatarsal bone to the most medial point overlying the capitulum of the first metatarsal bone, in a line perpendicular to the long axis of the foot

#### SOURCES OF ERROR IN MAKING MEASUREMENTS

##### 1 *Experimental errors*

Experimental errors in measuring were computed in the following manner. A series of ten measurements of the dimension in question was made on each of a group of infants. The scale on the instrument used was concealed from the anthropometrist by a slip of paper and readings were made and recorded by a second person. The average for each series was calculated, and deviations from the average recorded. All the deviations on all the infants were then grouped and the standard deviations computed. The errors worked out in this manner for the various dimensions are shown in table 1.

### *2 Errors due to thickness of subcutaneous tissues*

Since all measurements, excepting breadth of nose, length of ear, and the eye measurements, were taken from bony or cartilaginous points, errors due to nutritional status were minimal. Todd (10) found no change in the biliac diameter of a group of adult cadavers following the injection of formalin. Further measurements, after all the soft tissues had been removed, showed a diminution of 4.7 and 2.8 mm for male white and male negro cadavers, respectively. These differences amount to less than 2 per cent. The changes reported in our series are well outside of this error.

TABLE 1  
*Experimental errors in making measurements*

Dimension	Number of patients	Number of measurements	Average	Standard deviation	Coefficient of variation
Total body length	14	123	598	2.2	0.4
Sitting height	18	162	449	2.3	0.5
Diameter of face (bimolar)	11	110	86.1	1.03	1.2
Bicristal diameter	14	136	102.0	1.35	1.3
Biacromial diameter	20	206	147.0	1.46	1.0
Circumference of thorax at nipples	13	126	379.7	2.43	0.6

### *3 Variations in muscle tone*

Since the measurement of total body length is made by placing the child on the measuring board and stretching him as much as possible, it is conceivable that infants with good muscle tone might be more difficult to stretch to their full body length and so would appear shorter than infants with poor muscle tone. Such variations might well be selective, healthy infants having better muscle tone and consequently appearing shorter than sick infants or those poorly nourished. This source of error was investigated in the following way. Since differences in muscle tone are manifested principally by the degree of flexion at the knee, the difference between the total length of the lower extremity as measured from the anterior superior iliac spine to the sole and as computed from the sum of the thigh length and the tibia-sole length was determined in each of a number of infants. Groups of

infants were then compared as to the source of material, sex, and the presence of illness. The results are shown in table 2.

The percentage reduction in total body length varies from 0.7 to 0.9 per cent. It does not vary significantly for the different groups of babies.

TABLE 2  
Reduction in total body length due to flexion at the knee

Material	Number of patients	Average total body length	Average difference lower extremity (calculated minus observed)	Percentage reduction in total body length due to flexion at knee
Healthy infants, Fifth Avenue Hospital	52	633	5.7	0.9
Healthy infants, Bellevue Hospital.	46	603	4.3	0.7
Newborns, male	108	505	4.4	0.9
Newborns female	51	499	4.7	0.9
Infants with acute intestinal intoxication	21	567	4.2	0.7

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## BODY BUILD IN INFANTS

### II THE PROPORTIONS OF THE EXTERNAL DIMENSIONS OF THE HEALTHY INFANT DURING THE FIRST YEAR OF LIFE

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Measurements of the external dimensions of healthy infants were made in order to establish curves of central tendency with which to compare sick infants. This report comprises a description of the methods employed for statistical analysis and for the presentation of data and a comparison of 2 groups of healthy infants from different social environments.

This series consists of over 18,000 measurements on 646 infants under 1 year of age of whom 303 were males and 343 females. In addition more than 1200 newborn infants have been measured. Data on these are not reported here.

The infants used in this series were healthy in the sense in which this term is generally used, i.e. they were not suffering from acute or chronic illness nor were they strikingly underweight.

Only infants of Caucasian antecedents have been included. The technique for making measurements of the external dimensions of infants has been described in detail in the preceding paper (1).

Except in occasional instances the entire series of measurements (30 in all) was made on each patient. Measurements of the head during the first 3 days of life have not been included in the graphs or calculations because of distortion due to head molding at birth. A series of head measurements on infants during the second week of life has been substituted for those omitted. Chest measurements during the first 3 days of life have been excluded because of the fluctuations in size incident to the establishment of the respiratory function in the newborn (2).

This series is made up of 2 groups of infants which will be designated as Group A and Group B

#### DESCRIPTION OF GROUP A INFANTS

Group A consists of 475 infants, most of whom were born at Fifth Avenue Hospital and observed repeatedly in a special small number of patients from private practice (25 infants 42 times) are included in this group

With few exceptions the income of the parents varied only ranging between \$30 and \$35 per week (in 1929)

Most of the infants were partially or completely breast-fed for 3 to 6 months. All patients from private practice and attending regularly the special clinic at the Fifth Avenue received cod liver oil and orange juice after the age of 3 months, yolk, and vegetable or cereal by the fourth month

All infants used in this study were born in the United States in order to study the influence of race on the proportions of the dimensions of the body. Group A infants were divided into three racial groups: British descent, North European, consisting of Irish, Scottish, German, Scandinavian and Finnish (234 infants), Central European, consisting of French, Hungarian, Rumanian, Polish, Czechoslovakian and Austrian (20 infants), Mediterranean, consisting of Southern Greek, Turkish and Spanish (126 infants), and infants of Eastern European descent (106 infants). In a small group the race could not be ascertained with any degree of accuracy.

The dimensions of the infants in each of the 4 groups were compared, in relation to body length, with the total group by means of scatter diagrams for cephalic breadth, diameter of face, bicondylar diameter, circumference of thorax, biacromial diameter, bicristal diameter and length of radius. In no instance were differences in the proportions of the external dimensions apparent.

In table 1 the proportions of several dimensions to total body length are compared for North European, Jewish and Mediterranean groups. Averages, standard deviations and probable errors are given in relation to total body length. Differences in the various racial groups and the probable errors of the differences are also included. The differences are small.

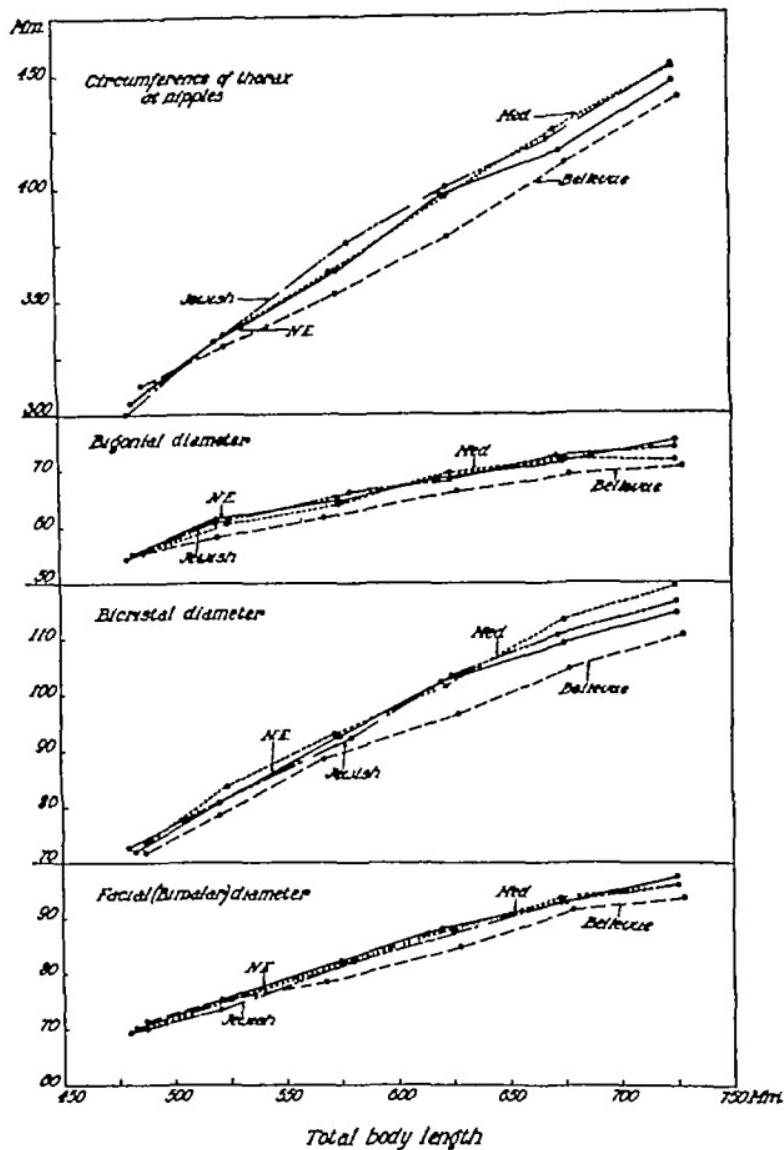


CHART 1 THE INFLUENCE OF RACE ON VARIOUS EXTERNAL DIMENSIONS OF INFANTS DURING THE FIRST YEAR OF LIFE IN RELATION TO TOTAL BODY LENGTH

The line marked N.E connects the average points for infants of North European parentage, Med, infants of Mediterranean parentage, etc.

*The influence of race on the proportions of the external dimensions of healthy infants from the Fifth Avenue Hospital*

North European				Jewish				Mediterranean			
Number of cases	Average and probable error	Standard deviation	Number of cases	Average and probable error	Standard deviation	Number of cases	Average and probable error	Standard deviation	mm.	mm.	mm.
Total body length											
24	482 ± 1.95	14.20	7	479 ± 2.84	11.20	17	488 ± 1.34	8.20			
69	522 ± 1.12	13.65	27	521 ± 1.72	13.25	32	526 ± 1.51	12.65			
43	576 ± 1.41	13.85	18	581 ± 1.51	9.50	19	573 ± 2.16	13.95			
30	621 ± 1.51	12.25	17	625 ± 1.96	11.95	22	623 ± 2.40	16.65			
48	674 ± 1.41	14.55	19	670 ± 2.14	13.95	23	673 ± 1.81	12.95			
20	724 ± 2.41	16.00	18	723 ± 2.26	14.25	13	725 ± 3.36	17.95			
North European				Jewish				North European minus Jewish			
Range of total body length	Average and probable error	Standard deviation	Average and probable error	Standard deviation	Average and probable error	Standard deviation	Difference and probable error (difference)	Difference and probable error divided by probable error (difference)	Difference and probable error divided by probable error (difference)	Difference and probable error divided by probable error (difference)	Jewish minus Mediterranean
mm.				mm.				mm.			
450-499	69.9 ± 0.37	2.72	69.4 ± 0.53	2.10	70.0 ± 0.45	2.77	0.5 ± 0.65	0.8	-0.1 ± 0.58	-0.2	-0.6 ± 0.69
500-549	75.1 ± 0.30	3.74	73.3 ± 0.48	3.68	75.2 ± 0.38	3.22	1.8 ± 0.57	3.1	-0.1 ± 0.48	-0.2	-1.9 ± 0.61
550-599	82.2 ± 0.52	5.06	82.1 ± 0.77	4.86	81.7 ± 0.55	3.58	0.1 ± 0.93	0.1	0.5 ± 0.76	0.7	0.4 ± 0.94
600-649	88.1 ± 0.49	3.99	87.5 ± 0.71	4.31	87.9 ± 0.52	3.64	0.6 ± 0.86	0.7	0.2 ± 0.71	0.3	-0.4 ± 0.88
650-699	92.8 ± 0.39	4.02	92.7 ± 0.74	4.81	93.7 ± 0.47	3.35	0.1 ± 0.84	0.1	-0.9 ± 0.61	-1.5	-1.0 ± 0.88
700-749	97.5 ± 0.57	3.80	96.0 ± 0.42	2.62	95.9 ± 0.52	2.76	1.5 ± 0.71	2.1	1.6 ± 0.77	2.1	0.1 ± 0.68

## Consequence of the theory at simple

4450-499	306	0 ± 1	55	11	30	299	6 ± 2	31	9	10	309	8 ± 1	79	10	95	6 ± 2	78	2	3	-3 ± 2	37	-1	6	-10 ± 2	92	-3	5		
5000-549	331	7 ± 1	38	17	10	331	0 ± 1	91	14	70	335	1 ± 1	89	15	85	0 ± 2	36	0	3	-3 ± 2	34	-1	5	-4 ± 1	69	-1	5		
5550-599	366	0 ± 1	90	18	65	376	7 ± 2	70	17	05	367	5 ± 2	64	17	00	-10 ± 3	30	-3	2	-1 ± 3	25	-0	5	9 ± 2	77	2	4		
6000-649	396	8 ± 2	18	17	70	399	3 ± 2	82	17	20	396	4 ± 2	64	18	40	-5 ± 3	56	-0	7	0 ± 4	42	0	1	2 ± 9	86	0	8		
6550-699	416	3 ± 1	71	17	55	421	5 ± 2	25	14	60	426	4 ± 1	36	9	70	-5 ± 2	83	-1	8	-10 ± 1	22	18	-4	5	-4 ± 9	63	-1	8	
7000-749	445	0 ± 2	44	16	20	452	8 ± 3	57	22	45	446	4 ± 4	46	23	85	-7 ± 8	44	-32	-1	8	-1 ± 4	55	0	3	6 ± 4	55	69	1	1

Bicentral diameter

4450-499	71	8 ± 0.42	3.04	72.7 ± 0.60	2.36	73.5 ± 0.51	3.08	-0.9 ± 0.74	-1.2	-1.7 ± 0.66	-2.6	-0.8 ± 0.79	-1.0
5000-549	80	5 ± 0.57	7.02	80.6 ± 0.86	6.64	83.5 ± 0.60	5.02	-0.1 ± 1.03	-0.1	-3.0 ± 0.83	-3.6	-2.9 ± 1.05	-2.8
5550-599	92	5 ± 0.59	5.86	92.2 ± 1.19	7.48	93.0 ± 0.83	5.34	0.3 ± 1.33	0.2	-0.5 ± 1.02	-0.4	-0.8 ± 1.45	-0.6
5900-649	102	1 ± 0.66	5.40	103.0 ± 0.93	5.68	101.3 ± 1.14	7.94	-0.9 ± 1.14	-0.8	0.8 ± 1.32	0.6	1.7 ± 1.47	1.2
5550-699	109	1 ± 0.68	7.06	110.5 ± 1.03	6.66	113.2 ± 0.71	5.04	-1.4 ± 1.23	-1.1	-4.1 ± 0.98	-4.2	-2.7 ± 1.25	-2.2
700-749	114	5 ± 1.01	6.72	116.5 ± 0.85	5.34	119.4 ± 0.82	4.42	-2.0 ± 1.32	-1.5	-4.9 ± 1.30	-3.8	-2.9 ± 1.18	-2.5

from one another irregularly. These results are illustrated in chart 1. The average lines for the Bellevue Hospital infants are consistently lower than the lines for each of the racial groups of Fifth Avenue Hospital infants.

In this series, therefore, no significant differences were apparent in the proportions of the various body dimensions to total body length in the three racial groups studied. It is possible that, in a larger series, differences might be found. The series is sufficiently large, however, to show that an environmental factor may influence body build.

All averages were calculated separately for sex. The males are significantly larger, relative to stature, in the hand and foot dimensions, and in the upper facial height. These differences have been treated statistically, in a manner similar to that used for comparison of infants of various racial groups, but calculations are not included in this report.

#### DESCRIPTION OF GROUP B INFANTS

Group B consists of 171 healthy infants admitted to the wards of Bellevue Hospital either as foundlings or because of illness of the mothers.

The incomes of the parents in the Bellevue district are very small, ranging between \$15 and \$22 per week (in 1929). Unemployment is common.

There was no control of the diets before admission to the hospital. Most of the infants in the Bellevue district are artificially fed from early life. The intake of energy-yielding foods is often inadequate. Small amounts of orange juice are given, but only rarely cod liver oil.

It was not possible to ascertain the descent of most of the infants because of the large proportion of foundlings. The healthy Bellevue infants are mainly of Irish descent.

#### GROWTH IN WEIGHT DURING THE FIRST YEAR OF LIFE

In chart 2 the weights of Group A and Group B infants are compared in relation to age during the first year of life. Age in weeks is represented as the abscissa, weight as the ordinate. The continuous line is the weight-age curve given by Holt and Howland for healthy

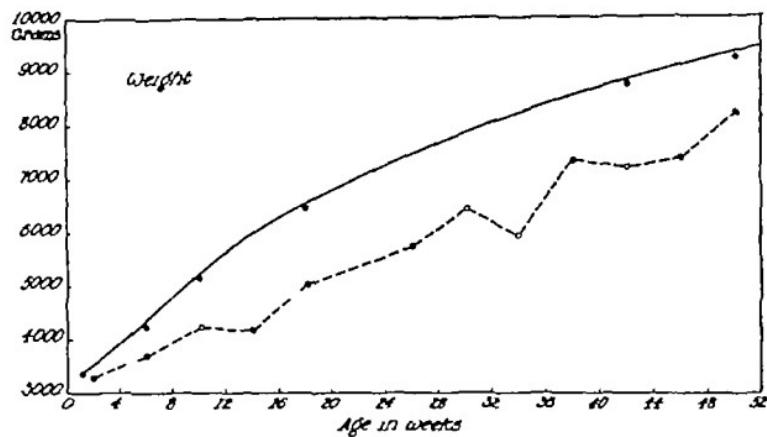


CHART 2 WEIGHT CURVE DURING THE FIRST YEAR OF LIFE

The continuous line is the age weight curve for healthy infants given by Holt and Howland. The dots represent averages for the Fifth Avenue Hospital infants, circles connected by the broken line, Bellevue Hospital infants.

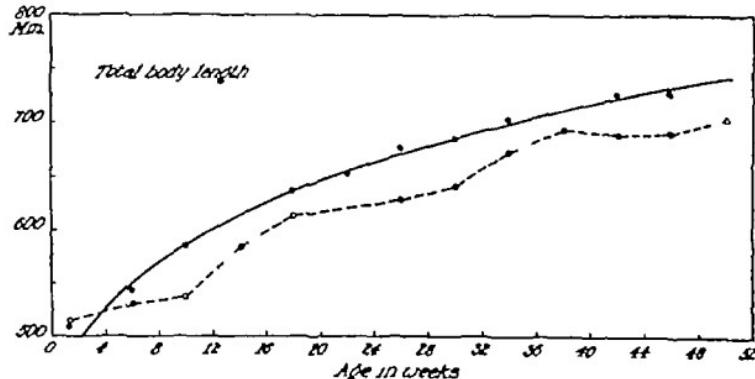


CHART 3 GROWTH OF TOTAL BODY LENGTH DURING THE FIRST YEAR OF LIFE

The continuous line is the curve of central tendency calculated according to the formula given in the text. The dots represent averages for the Fifth Avenue Hospital infants, the circles connected by the broken line, Bellevue Hospital infants.

infants (3). The dots represent average weights for the Group A infants, the circles connected by the dotted line averages for the Group B infants. The Group A infants follow the normal curve closely

The Group B infants, after the first month of life, fall far below the irregularity in the distribution of the averages for this group is due, in part to the small number of cases (no foundlings are included since the ages are unknown excepting those admitted during the new born period), and in part to a selective death rate, the thin babies dying at an earlier age than the heavier ones

#### GROWTH IN TOTAL BODY LENGTH DURING THE FIRST YEAR OF LIFE

In chart 3 the total body length of Group A and Group B infants are compared by age. Age in weeks is represented as the abscissa, total body length as the ordinate. The continuous line is the curve of central tendency for increase of total body length in relation to age during the first year of life for Group A infants. The dots represent averages of total body length for Group A infants, the circles, connected by the dotted line, averages for the Group B infants. After the second month of life the Group B infants fall below the curve of central tendency, indicating a retardation in growth of total body length.

The empirical formula for this curve, computed by the method of least squares, is,

$$L = 493 + 12T + 170 \log(0.25T + 0.5) \quad (1)$$

where  $L$  represents total body length in mm, and  $T$  age in weeks. From this formula and the empirical formulae given in table 2 for the relationship between the various external dimensions and total body length, empirical formulae for the relationship between various dimensions and age can be readily computed. Thus for facial diameter, where  $F$  represents facial diameter,

$$F = 0.107L + 20.4 \quad (2)$$

Transposing (2),

$$L = \frac{F - 20.4}{0.107} \quad (3)$$

Substituting,  $\frac{F - 20.4}{0.107}$  for  $L$  in (1) we have,

$$F = 73.3 + 0.128T + 18.20 \log(0.25T + 0.5) \quad (4)$$

HARRY BAKWIN AND ETHE MORIS BAKWIN

GROWTH OF THE EXTERNAL DIMENSIONS OF THE BODY IN RELATION  
TOTAL BODY LENGTH

The method used for presenting the data on growth of the external dimensions in relation to total body length is essentially the same as that used by Scammon and Calkins in their study of growth during the fetal period (4). The advantages of using total body length than sitting height as a base are discussed by Scammon and Calkins.

Scatter diagrams were constructed for each dimension with time represented as the abscissa and the dimension in question as the ordinate. The data for each dimension were then divided into six groups on the basis of stature, each group representing a 5 mm interval. Numerical averages for both stature and the dimension in question were calculated for each of the 6 groups and these averages were used in the derivation of empirical formulae for the curves of central tendency.

It appeared that the dimensions measured could be adequately represented by straight line curves when plotted in relation to total body length. Curves have been computed only for infants up to 750 mm in total body length.

Scatter diagrams and curves of central tendency for the relations in Group A infants, between total body length and diameter (bimolar), bigonial diameter, biacromial diameter, true circumference of thorax at the nipples and bircristal diameter are shown in chart 4. Dots represent measurements on female infants, crosses on males. The continuous lines are the curves of central tendency plotted according to the empirical formulae given in table 2.

In table 2 are shown the constants in the empirical formulae for the curves of central tendency of the dimensions listed in the first part of this series (1). The formulae, with the exception of those for diameter (bimolar), bigonial diameter, circumference of thorax at the nipples, and bircristal diameter were computed on the basis of measurements on 320 infants from the Fifth Avenue Hospital and 61 from Bellevue Hospital.

The compilation of these data was made originally on 320 healthy infants from the Fifth Avenue Hospital and 61 healthy infants from Bellevue Hospital. No significant differences were found in the proportions of the body dimensions of the two groups.

became apparent that they could not be treated together. In the meantime more measurements had been made at both hospitals and the original series, enlarged by the addition of the new measurements, has been used in this paper except when otherwise stated. Having two groups of infants from two hospitals,

TABLE 2

*Constants in empirical formulae ( $y = ax + b$ )<sup>\*</sup> for computing the curves of central tendency from total body length*

Dimension	Constants	
	a	b
Sitting height	0.545	71.6
Circumference of head	0.458	118
Cephalic length	0.127	55
Cephalic breadth	0.154	14.5
Diameter of face (bimolar)	0.107	20.4
Bigonal diameter	0.074	21.5
Upper facial height (nasion-prosthion)	0.039	12.4
Height of lower jaw	0.028	3.8
Height of nose	0.026	8.8
Breadth of nose	0.017	12.9
Inter-inner canthus diameter	0.016	14.1
Length of palpebral fissure	0.024	8.1
Length of ear	0.053	9.6
Breadth of ear	0.024	12.7
Circumference of thorax at the nipples	0.562	42
Biacromial diameter	0.239	-9.6
Bicristal diameter	0.168	-4.3
Length of humerus	0.164	2.3
Length of radius	0.116	12.1
Length of hand	0.095	16.2
Length of palm	0.055	8.7
Breadth of palm	0.043	9.8
Length of middle finger	0.040	13.5
Length of tibia	0.170	5.4
Length of thigh	0.247	-7.9
Height of foot	0.059	-5.8
Length of foot	0.121	15.8
Breadth of foot	0.053	4.0

\*  $y$  equals the linear dimension in question,  $x$  equals total body length,  $a$  and  $b$  are constants

an old and a new group, permitted various checks to be made. Thus, the differences noted below in the proportions of the external dimensions of Group A and Group B infants have been checked on two different groups from Bellevue Hospital and

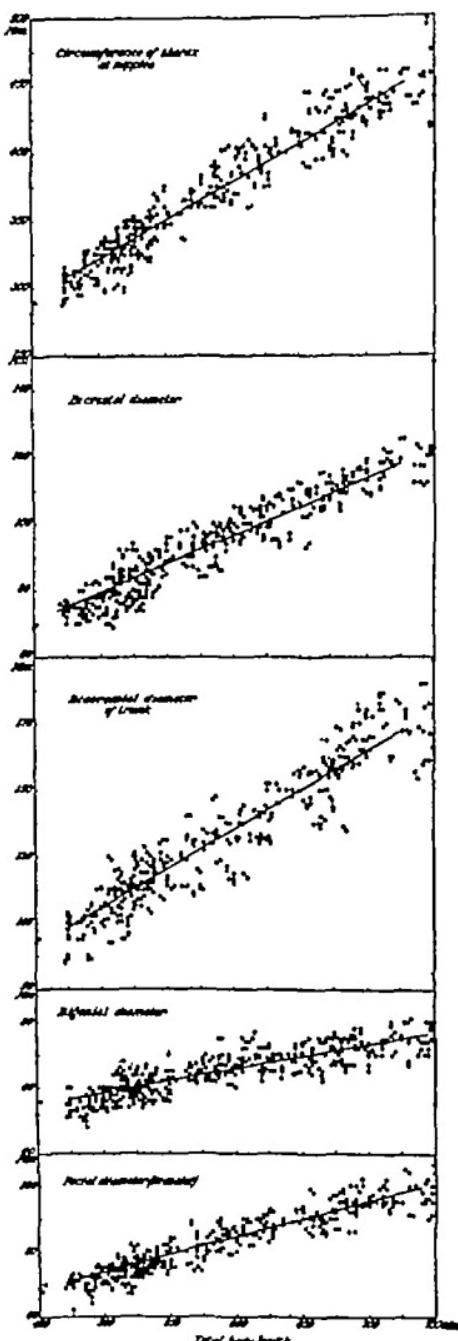


CHART 4 THE RELATIONSHIP BETWEEN TOTAL BODY LENGTH AND VARIOUS LINEAR DIMENSIONS DURING THE FIRST YEAR OF LIFE  
(FIFTH AVENUE HOSPITAL INFANTS)

Dots represent individual measurements on females, the circles on males. The continuous lines are the curves of central tendency plotted according to the formulae given in table 2

have been controlled by the absence of such changes when the new Fifth Avenue Hospital group has been compared with the old

COMPARISON OF THE PROPORTIONS OF THE EXTERNAL DIMENSIONS OF  
HEALTHY INFANTS FROM THE FIFTH AVENUE AND  
BELLEVUE HOSPITALS

The 171 infants in Group B were compared for various dimensions with the curves of central tendency for Group A by means of scatter diagrams. No differences were found for the relative lengths of the following dimensions—circumference of head, cephalic length, cephalic breadth, upper facial height, length of humerus, radius, femur and tibia. Differences were noted for the facial diameter (bimolar), the bigonial diameter, the circumference of the thorax, the bicristal diameter, and the biacromial diameter, the Group B infants in each instance tending to fall below the curves of central tendency, indicating that the Group B infants are relatively narrower in their transverse dimensions and chest than the Group A infants. These differences are not marked for infants below 550 mm. in height, beyond this the difference tends to increase, approximating in most instances from 5 to 7 times the probable error. These results are shown in tables 3 and 4 and are illustrated graphically in charts 5 and 6. Tables 3 and 4 include standard deviations and probable errors for each of the dimensions at various levels of total body length.

When comparisons were made of the biacromial diameter, it was found that the new measurements were larger than the old, both for the Fifth Avenue Hospital and the Bellevue Hospital infants, owing, presumably, to an unconscious change in the technique of measuring. Separate calculations of the biacromial diameter have therefore been made for the new groups of infants only, and are shown in table 4 and chart 6.

Approximately fifty Bellevue Hospital infants, admitted when healthy and measured before the onset of illness, developed acute intestinal intoxication. These have not been included in the Group B series. As will be shown in a later paper, infants with this condition are smaller in their transverse diameters and chest circumference relative to stature, than are either Group A or Group B infants. Had these been included in the Bellevue group the discrepancy between it and the Fifth Avenue group would have been even greater.

TABLE 3

*Comparison of the proportions of the external dimensions of healthy infants from the Fifth Avenue and Bellevue Hospitals*

Class	Range of total body length	Fifth Avenue Hospital (A)			Bellevue Hospital (B)		
		Number of infants	Total body length average	Number of infants	Total body length average		
1	450-499	54	484	44	487		
2	500-549	135	524	40	521		
3	550-599	76	577	24	568		
4	600-649	72	624	22	628		
5	650-699	84	675	21	677		
6	700-749	54	725	20	728		

Class	Fifth Avenue Hospital (A)			Bellevue Hospital (B)			Difference Group A minus Group B and probable error (difference)	Difference divided by probable error (difference)
	Average and probable error	Standard deviation	Coefficient of variation	Average and probable error	Standard deviation	Coefficient of variation		
a. Diameter of face (bimolar)								
1	71.0 ± 0.28	3.03	4.2	70.8 ± 0.29	2.81	3.9	0.2 ± 0.40	0.5
2	75.3 ± 0.23	3.99	5.3	75.3 ± 0.44	4.20	5.5	0.0 ± 0.49	0.0
3	82.4 ± 0.34	4.38	5.3	78.3 ± 0.58	4.28	5.4	4.1 ± 0.67	6.1
4	88.1 ± 0.31	3.95	4.5	84.8 ± 0.42	2.89	3.4	3.3 ± 0.52	6.3
5	93.9 ± 0.37	5.07	5.4	91.4 ± 0.64	4.35	4.7	2.5 ± 0.74	3.4
6	97.4 ± 0.37	4.06	4.2	93.9 ± 0.48	3.15	3.3	3.5 ± 0.61	5.8
b. Bicondylar diameter								
1	55.6 ± 0.36	3.91	7.0	54.1 ± 0.28	2.76	5.1	1.5 ± 0.45	3.3
2	60.8 ± 0.28	4.78	7.7	58.0 ± 0.45	4.24	7.0	2.8 ± 0.53	5.3
3	65.4 ± 0.31	4.05	6.2	61.6 ± 0.62	4.48	7.3	3.8 ± 0.69	5.5
4	69.4 ± 0.31	3.79	5.4	66.2 ± 0.62	4.48	6.8	3.2 ± 0.69	4.7
5	71.8 ± 0.29	3.90	5.4	69.3 ± 0.47	3.17	4.5	2.5 ± 0.54	4.6
6	74.2 ± 0.37	4.09	5.5	70.8 ± 0.49	3.26	4.6	3.4 ± 0.61	5.6
c. Circumference of thorax at nipples								
1	308 ± 1.10	12.1	3.9	308 ± 1.30	12.1	3.2	0 ± 1.70	0.0
2	334 ± 0.93	16.1	4.8	329 ± 2.07	19.4	5.9	5 ± 2.27	2.2
3	369 ± 1.48	19.2	5.2	353 ± 2.51	18.9	5.4	16 ± 2.91	5.5
4	397 ± 1.42	18.4	4.6	378 ± 2.04	14.5	3.8	19 ± 2.56	7.2
5	422 ± 1.37	18.5	4.4	410 ± 1.43	9.6	2.3	12 ± 1.98	6.1
6	450 ± 1.87	20.7	4.6	438 ± 2.80	18.5	4.2	12 ± 3.37	3.5

TABLE 3—Concluded

Class	Fifth Avenue Hospital (A)			Bellevue Hospital (B)			Difference Group A minus Group B and probable error (difference)	Difference divided by probable error (difference)
	Average and probable error	Standard deviation	Coefficient of variation	Average and probable error	Standard deviation	Coefficient of variation		
d Bicristal diameter								
	mm	mm	per cent	mm	mm	per cent	mm	
1	75.4 ± 0.40	4.30	5.7	71.8 ± 0.70	6.86	9.5	3.6 ± 0.81	4.4
2	81.6 ± 0.40	6.72	8.2	78.3 ± 0.74	6.88	8.8	3.3 ± 0.84	3.9
3	93.5 ± 0.51	6.54	7.0	88.7 ± 0.91	6.68	7.5	4.8 ± 1.04	4.6
4	101.7 ± 0.53	6.60	6.5	96.4 ± 0.98	6.78	7.0	5.3 ± 1.11	4.8
5	110.7 ± 0.53	7.20	6.5	104.6 ± 0.76	5.12	4.9	6.1 ± 0.93	6.6
6	117.2 ± 0.59	6.56	5.0	110.3 ± 0.94	6.22	5.6	6.9 ± 1.11	6.2

TABLE 4

*Comparison of the proportions of the biacromial diameters of healthy infants from the Fifth Avenue and Bellevue Hospitals*

Range of total body length	Fifth Avenue Hospital (A)			Bellevue Hospital (B)			Difference Group A minus Group B and probable error (difference)	Difference divided by probable error (difference)
	Number of cases	Average and probable error	Standard deviation	Number of cases	Average and probable error	Standard deviation		
<i>mm</i>								
450-499	23	115.2 ± 0.65	4.66	12	116.0 ± 1.10	5.66	-0.8 ± 1.27	-0.7
500-549	69	124.8 ± 0.47	5.78	42	121.3 ± 0.62	6.00	3.5 ± 0.78	4.5
550-599	60	136.4 ± 0.54	6.16	22	127.8 ± 0.88	6.10	8.6 ± 1.03	8.3
600-649	54	147.1 ± 0.63	6.84	22	145.5 ± 0.84	5.86	1.6 ± 1.05	1.5
650-699	66	160.9 ± 0.49	5.98	23	157.0 ± 0.95	6.72	3.9 ± 1.07	3.6
700-749	55	172.7 ± 0.79	8.68	31	169.4 ± 0.79	6.52	3.3 ± 1.12	2.9

The difference between the two groups of infants are not dependent on differences in race, since as shown above, this was found to be without influence on the proportions of the body dimensions in this series. Nor does it seem likely that technical errors in making measurements, dependent on the greater amount of subcutaneous fat in the Group A infants, can account for the differences found. The factor of muscle tonus variations has also been considered (1) and has

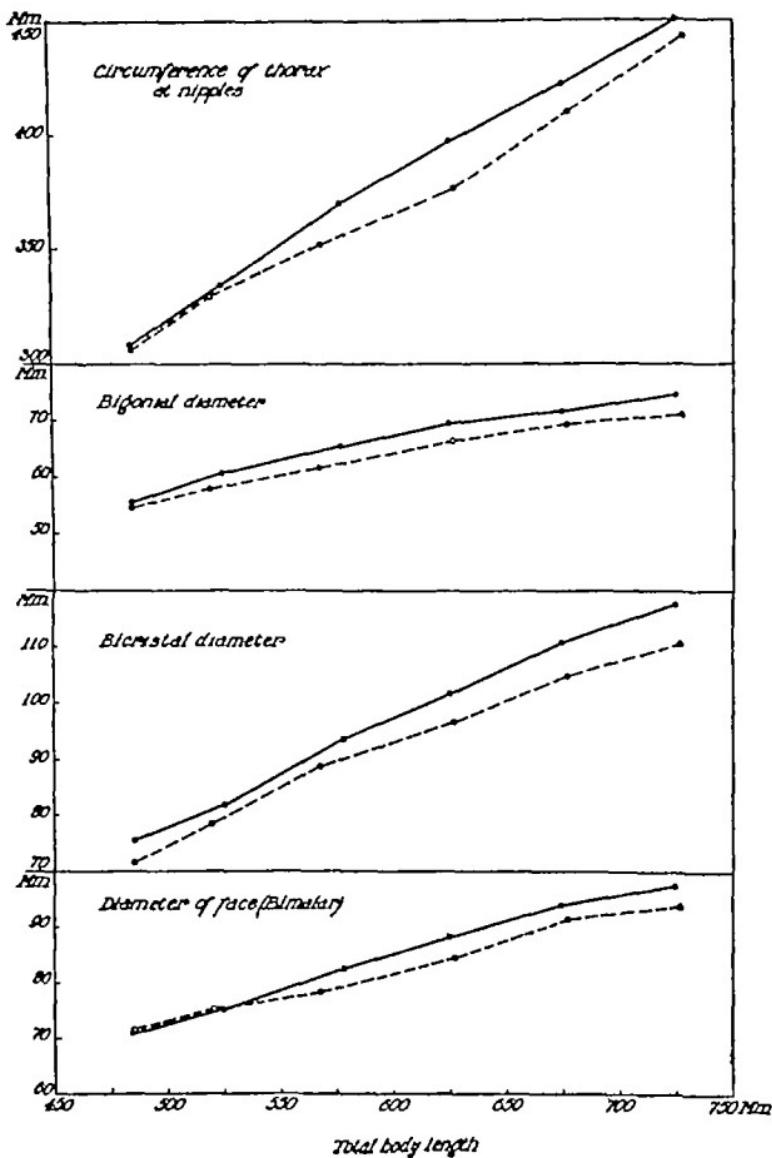


CHART 5 COMPARISON OF THE PROPORTIONS OF VARIOUS DIMENSIONS OF FIFTH AVENUE AND BELLEVUE HOSPITAL INFANTS

The solid lines connect the average points for Fifth Avenue Hospital infants, the broken lines the Bellevue Hospital infants.

been found to be without influence in explaining differences in the proportions of the dimensions of the two groups

#### COMMENT

Accompanying the delay in weight gain of infants from poor homes there is a retardation in the growth of the linear measurements. This is more marked for the transverse diameters, (facial diameter, bigonial diameter, biacromial diameter and bicristal diameter) and for

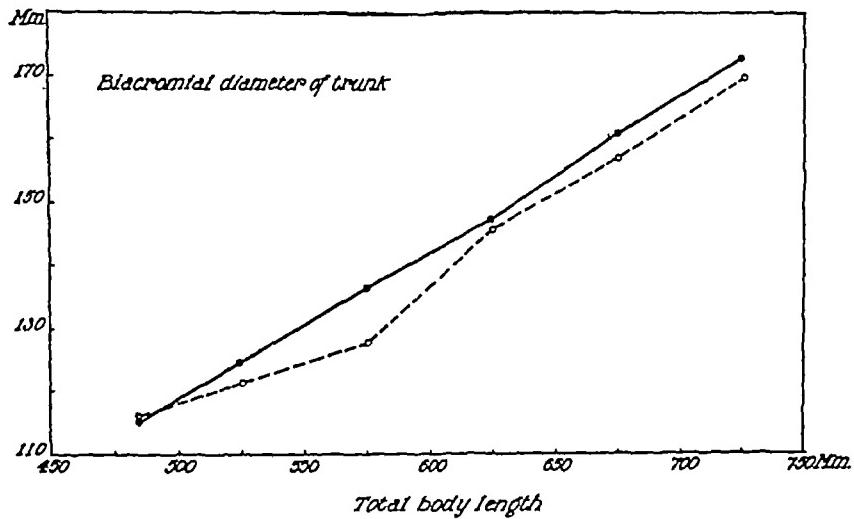


CHART 6 COMPARISON OF THE PROPORTIONS OF THE BIACROMIAL DIAMETER OF FIFTH AVENUE AND BELLEVUE HOSPITAL INFANTS

The solid lines connect the average points for Fifth Avenue Hospital infants, the broken lines the Bellevue Hospital infants

the circumference of the thorax, than for the cephalocaudal or vertical dimensions (total body length, upper facial height, length of humerus, radius, femur and tibia). There results a change in the proportions of the external dimensions, the infants from poor families becoming relatively narrower than infants from families of moderate income. It seems reasonable to assume that this change is dependent on the environmental factors implied in the difference in the social condition. The factors influencing growth and development at this age, such as diet, atmospheric conditions and infection all vary with the social condition.

Racial differences (Caucasians only), if they play any part in determining body build in infants, were too small to be apparent in this study.

It is not possible to say whether these changes in body build are permanent. That they are profound and clinically significant will be shown in the paper on the relationship between body build and disease during infancy.

The changes in body build here noted recall the well known observations of Boas (5), who found a change in body build of American born children of immigrants.

#### SUMMARY

1 Measurements of the external dimensions of 2 groups of healthy infants under 1 year have been made.

2 One group was observed, for the most part, at a special clinic at the Fifth Avenue Hospital. The infants came from families of moderate income and received diets adequate in the known essential food substances at an early age. The second group, many of whom were foundlings, was observed at Bellevue Hospital. The infants came from very poor families and their diets were probably inadequate in several of the known essential food substances as well as in the total intake of energy-yielding foods.

3 After the first month and at least until the end of the first year of life (calculations have been made only for the first year of life) the infants from the poor homes (Bellevue district) weighed less and were shorter in stature than those from the better homes (Fifth Avenue Hospital patients).

4 Using total body length as a base line empirical formulae have been computed for curves of central tendency for the various external dimensions of the bodies of the infants from homes of moderate incomes.

5 Above 550 mm in body length infants from the poorer homes are smaller, relative to total body length, in their transverse diameters and in the circumference of the thorax, than are infants from better homes. The retardation in the growth of the external dimensions, accompanying the delay in weight gain, is greater for the transverse than for the cephalocaudal, or vertical dimensions.

6 No differences in the proportion of the external dimensions of the body due to race (Caucasians only) were apparent in this series

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## BODY BUILD IN INFANTS

### III BODY BUILD IN DISEASE

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Measurements of the external dimensions of sick infants were made and compared with the values obtained in healthy infants (1)

The technique employed for making the measurements has been described in a previous paper (2)

#### DESCRIPTION OF MATERIAL

Only sick infants observed at Bellevue Hospital are reported here and hence, comparisons are made only with the healthy infants admitted to Bellevue Hospital

a *Acute intestinal intoxication* Acute intestinal intoxication is here used to designate a type of reaction in infants characterized by somnolence alternating with periods of hyper-irritability and at times convulsions, cyanosis, hyperpnea, oliguria or anuria, and evidence of dehydration There is usually fever and an associated infection Diarrhea and vomiting may or may not be present There are no characteristic gross anatomical changes except, perhaps, in the liver Most of the infants here reported were ill during the winter months

There were 133 infants in this group of whom 64 were males and 69 females Forty-four (33 per cent) were admitted to the hospital and measured before the onset of illness Forty infants (30 per cent) were foundlings There were 78 deaths (59 per cent)

b *Tetany* By tetany in infants is meant a metabolic disorder resulting from deprivation of vitamin D or sunlight, characterized by a tendency to convulsions and spasms of various kinds The calcium concentration in the serum is regularly diminished and there is a change in the reaction to galvanic stimulation It may be cured by the administration of vitamin D or ultraviolet radiant energy

Only infants in whom the serum calcium concentration was below 8.0 mgm per cent are included in this group. The diagnosis was confirmed in most instances with the electrical reactions. There were 45 infants in this group of whom 28 were males and 17 females.

*c Eczema* Only infants with typical facial eczema are included. There were 59 infants in this group of whom 41 were males and 18 females.

TABLE 1

*Comparison of the total body lengths of healthy infants and sick infants at Bellevue Hospital*

Age weeks	Healthy		Acute intestinal intoxication		Tetany		Eczema	
	Number of cases	Average total body length mm	Number of cases	Average total body length mm	Number of cases	Average total body length mm	Number of cases	Average total body length mm
0-3	31	516	12	507				
4-7	19	532	34	523				
8-11	13	537	15	516	4	531	5	576
12-15	9	594	20	563			4	618
16-19	8	612	10	601	5	592	5	641
20-23			11	619	9	605	8	650
24-27	6	628	8	621	3	622	11	657
28-31	9	640	2	642	7	645	4	667
32-35	3	673	4	630	8	672	4	653
36-39	5	693	5	656	3	650		
40-43	7	688	2	653				
44-47	3	690	4	708	5	693		
48-51	6	702	2	673				

#### EXPLANATION OF CHARTS

A number of charts, illustrating the differences in the proportions of the external dimensions of infants in health and disease, are shown. In each chart total body length is represented as the abscissa, the dimension in question as the ordinate. The broken lines connect the average points for the healthy Bellevue Hospital infants. Circles indicate infants with acute intestinal intoxication, dots infants with tetany, and crosses infants with eczema.

#### RESULTS

In table 1, the total body length of healthy and sick infants at Bellevue Hospital are compared in relation to age. Infants with acute

intestinal intoxication are, on the average, shorter than the healthy infants, while infants with eczema are longer. The infants with

TABLE 2

*Comparison of infants with acute intestinal intoxication and healthy infants from Bellevue Hospital*

Range of total body length	Healthy Bellevue Hospital infants		Infants with intestinal intoxication			Difference healthy infants minus infants with intestinal intoxication and probable error (difference)	Difference divided by probable error (difference)
	Average and probable error	Stand devia tion	Number of cases	Average and probable error	Stand devia tion		
a. Diameter of face (bimolar)							
mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
450-499	70.8 ± 0.29	2.81	16	68.3 ± 0.53	3.15	2.5 ± 0.60	4.2
500-549	75.3 ± 0.44	4.20	53	72.6 ± 0.38	4.05	2.7 ± 0.56	4.8
550-599	78.3 ± 0.58	4.28	27	78.3 ± 0.38	2.96	0.0 ± 0.69	0.0
600-649	84.8 ± 0.42	2.89	27	82.9 ± 0.71	5.49	1.9 ± 0.83	2.3
650-699	91.4 ± 0.64	4.35	10	86.6 ± 0.47	2.21	4.8 ± 0.79	6.1
b. Bigonial diameter							
mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
450-499	54.1 ± 0.28	2.76	16	53.2 ± 0.40	2.37	0.9 ± 0.49	1.8
500-549	58.0 ± 0.45	4.24	53	56.8 ± 0.26	2.98	1.2 ± 0.52	2.3
550-599	61.6 ± 0.62	4.48	28	60.9 ± 0.38	2.98	0.7 ± 0.73	1.0
600-649	66.2 ± 0.62	4.48	27	64.7 ± 0.63	4.84	1.5 ± 0.88	1.7
650-699	69.3 ± 0.47	3.17	10	67.1 ± 0.44	2.05	2.2 ± 0.64	3.4
c. Circumference of thorax at nipples							
mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
450-499	308 ± 1.30	12.1	13	295 ± 1.90	11.25	13 ± 2.30	5.7
500-549	329 ± 2.07	19.4	52	320 ± 1.61	17.10	9 ± 2.62	3.5
550-599	353 ± 2.51	18.9	27	344 ± 2.64	20.80	9 ± 3.64	2.5
600-649	378 ± 2.04	14.5	26	369 ± 3.00	23.05	9 ± 3.63	2.5
650-699	410 ± 1.43	9.6	10	398 ± 3.68	17.20	12 ± 3.95	3.0
d. Bicristal diameter							
mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
450-499	71.9 ± 0.70	6.86	16	74.4 ± 0.62	3.68	-2.6 ± 0.94	-2.8
500-549	78.3 ± 0.74	6.88	53	81.3 ± 0.40	4.56	-3.0 ± 0.84	-3.6
550-599	88.7 ± 0.91	6.68	26	88.1 ± 0.75	5.86	0.6 ± 1.18	0.5
600-649	96.4 ± 0.98	6.78	27	97.6 ± 0.89	6.84	-1.2 ± 1.32	-0.9
650-699	104.6 ± 0.76	5.12	10	103.4 ± 0.97	4.54	1.2 ± 1.23	1.0

tetany are spread over so wide an age range that the averages have little value.

TABLE 3  
*Comparison of infants with tetany and healthy infants from Bellevue Hospital*

Range of total body length	Healthy Bellevue Hospital infants		Infants with tetany			Difference healthy infants minus infants with tetany and probable error (difference)	Difference divided by probable error (difference)
	Average and probable error	Stand ard devia tion	Num ber of cases	Average and probable error	Stand ard devia tion		
a Diameter of face (bimolar)							
mm	mm	mm	mm	mm	mm	mm	mm
600-649	84.8 ± 0.42	2.89	13	93.9 ± 0.53	2.81	-9.1 ± 0.68	-13.4
650-699	91.4 ± 0.64	4.35	18	96.1 ± 1.02	6.42	-4.7 ± 1.20	-3.9
b Bigonial diameter							
600-649	66.2 ± 0.62	4.48	14	71.9 ± 0.88	4.92	-5.7 ± 1.08	-5.3
650-699	69.3 ± 0.47	3.17	18	73.8 ± 0.78	4.91	-4.5 ± 0.91	-4.9
c Circumference of thorax at nipples							
600-649	378.2 ± 2.04	14.5	15	401.4 ± 4.47	25.60	-23.4 ± 4.91	-4.7
650-699	410.1 ± 1.43	9.6	17	421.3 ± 3.16	19.45	-11.3 ± 3.47	-3.2
d Bicristal diameter							
600-649	96.4 ± 0.98	6.78	15	101.4 ± 0.82	4.68	-5.0 ± 1.28	-3.9
650-699	104.6 ± 0.76	5.12	18	111.3 ± 0.76	4.76	-6.7 ± 1.08	-6.2

TABLE 4  
*Comparison of infants with eczema and healthy infants from Bellevue Hospital*

Range of total body length	Healthy Bellevue Hospital infants		Infants with eczema			Difference healthy infants minus infants with eczema and probable error (difference)	Difference divided by probable error (difference)
	Average and probable error	Stand ard devia tion	Num ber of cases	Average and probable error	Stand ard devia tion		
a Diameter of face (bimolar)							
mm	mm	mm	mm	mm	mm	mm	mm
600-649	84.8 ± 0.42	2.89	19	89.5 ± 0.61	3.94	-4.7 ± 0.74	-6.4
650-699	91.4 ± 0.64	4.35	20	93.8 ± 0.59	3.91	-2.4 ± 0.87	-2.8
b Bigonial diameter							
600-649	66.2 ± 0.62	4.48	17	68.5 ± 0.55	3.39	-2.3 ± 0.83	-2.8
650-699	69.3 ± 0.47	3.17	20	73.3 ± 0.65	4.28	-4.0 ± 0.80	-5.0
c Circumference of thorax at nipples							
600-649	378.2 ± 2.04	14.50	19	393.2 ± 2.64	17.05	-15.3 ± 3.34	-4.5
650-699	410.1 ± 1.43	9.60	20	416.2 ± 2.44	16.10	-6.2 ± 2.81	-2.1
d Bicristal diameter							
600-649	96.4 ± 0.98	6.78	19	102.9 ± 0.60	3.87	-6.5 ± 1.15	-5.6
650-699	104.6 ± 0.76	5.12	19	111.2 ± 1.10	7.10	-6.6 ± 1.34	-4.9

In tables 2, 3 and 4, various external dimensions of healthy and sick infants are compared in relation to total body length. The distribution of the measurements on individual patients is illustrated in charts 1 to 4.

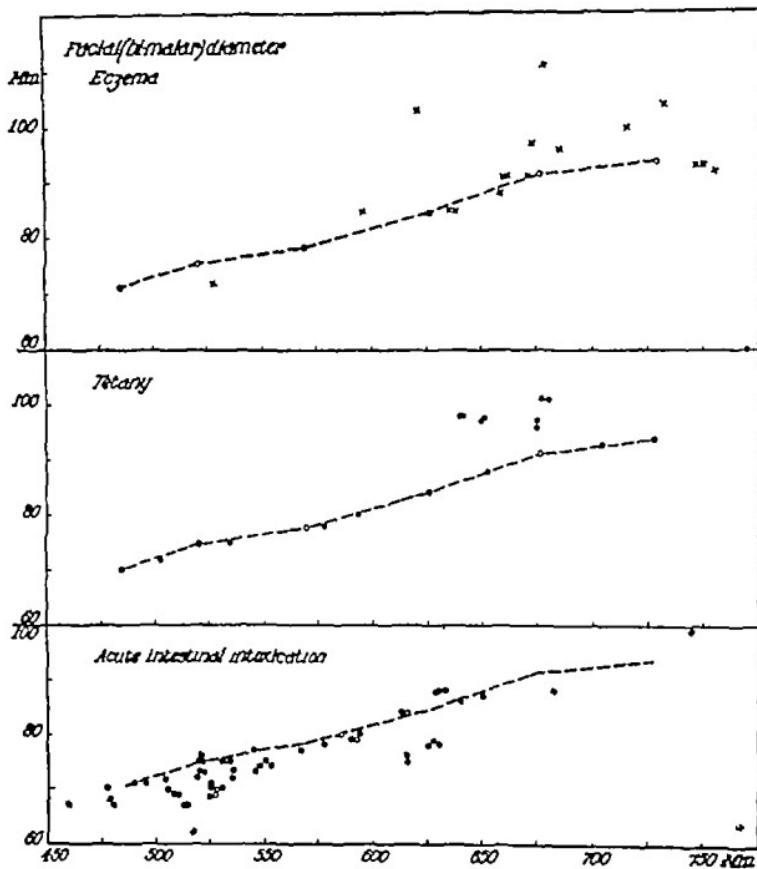


CHART 1 THE RELATIVE WIDTH OF THE FACE (BIMALAR DIAMETER) IN INFANTS WITH VARIOUS DISEASES

*Acute intestinal intoxication.* It is apparent from the charts that the circles, indicating patients with acute intestinal intoxication, tend to fall below the average lines for healthy infants for the diameter of face, the bigonial diameter and the circumference of the thorax. There is

no difference for the bicristal diameter. These differences, though not always large enough to be significant, are nevertheless regular, the averages for the infants with acute intestinal intoxication being constantly lower than those for the healthy infants with the exception of the bicristal diameter.

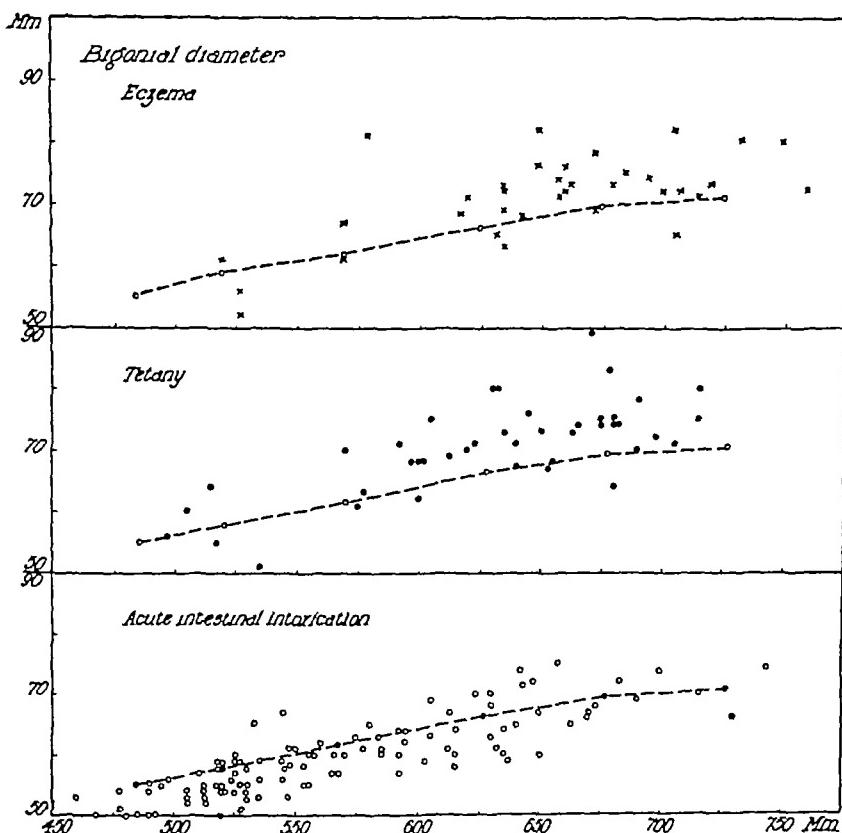


CHART 2 THE RELATIVE DIAMETER AT THE ANGLE OF THE JAWS (BIGNONIAL DIAMETER) IN INFANTS WITH VARIOUS DISEASES

*Tetany* The dots, indicating measurements on infants with tetany, tend to fall above the average lines for the healthy infants for all four dimensions shown. Differences from the healthy have been calculated for infants ranging from 600 to 649, and 650 to 699 mm in height only, since these were the only groups in which the number of cases was sufficiently large to make the results significant. Averages for the

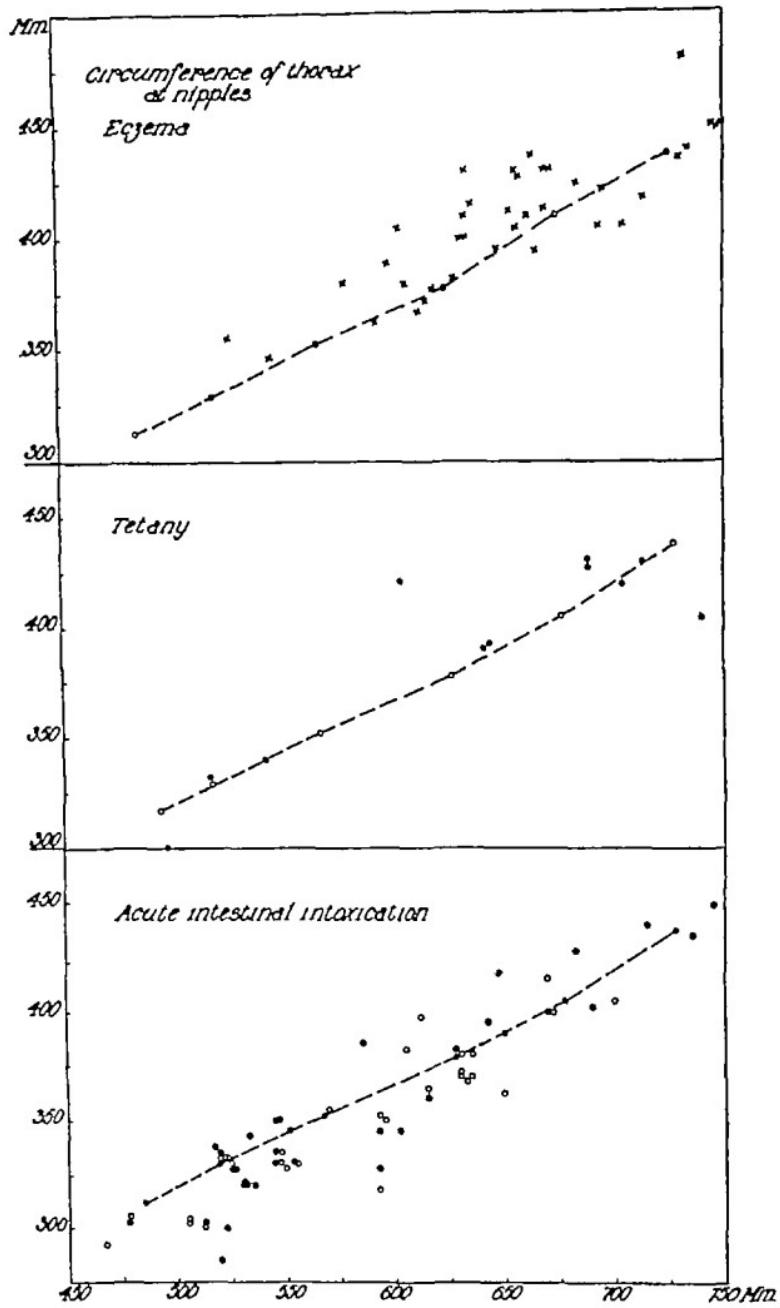


CHART 3 THE RELATIVE CIRCUMFERENCE OF THE THORAX IN INFANTS WITH VARIOUS DISEASES

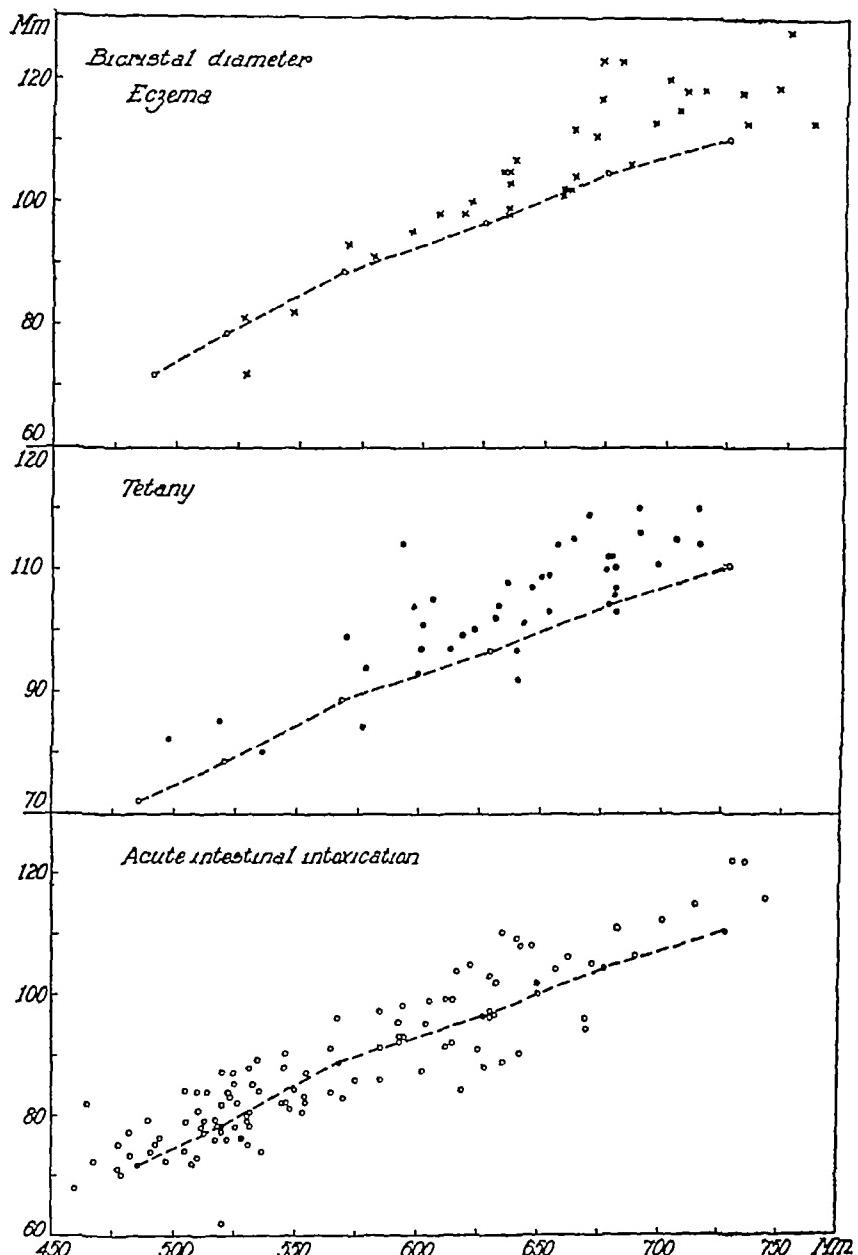


CHART 4 THE RELATIVE WIDTH OF THE HIPS (BICRISTAL DIAMETER) IN INFANTS WITH VARIOUS DISEASES

infants with tetany were regularly higher than for the healthy infants, the difference in all instances being greater than three times the probable error of the difference.

*Eczema* The crosses, indicating measurements on infants with eczema, tend to fall above the average lines for the healthy infants for all four dimensions shown. Differences from the healthy have been calculated for infants ranging from 600 to 649 and 650 to 699 mm in height only, since these were the only groups in which the number of cases was sufficiently large to make the results significant. Averages for the eczema infants were regularly higher than for the healthy infants, the differences in five of the eight groups being more than three times greater than the probable error of the difference.

#### SUMMARY

1 The proportions of the external dimensions of infants with various diseases were studied

2 Relative to total body length, infants with acute intestinal intoxication are smaller in their transverse dimensions (diameter of face, bigonial diameter) and in their chest circumference than are healthy infants from the same social environment.

3 Infants with tetany and infants with eczema are relatively larger in their transverse dimensions (diameter of face, bigonial diameter and bicristal diameter) and in their chest circumference than are healthy infants from the same social environment

We wish to express our sincere thanks to Dr Franz Boas for many helpful suggestions in the treatment of these data

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## THE MECHANICAL INFLUENCE OF THE PERICARDIUM UPON CARDIAC FUNCTION

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The action of the heart may be profoundly disturbed by alterations in the pressure maintained over its outer surface. Tamponade of the heart by an intrapericardial hemorrhage or effusion is the most familiar example and is such a classical phenomenon, both clinically and experimentally, that description would be superfluous. Adhesive pericarditis of the type called *concreto pericardii*, commonly termed Pick's disease, may produce many of the signs and symptoms of cardiac tamponade over a period of years. The essential lesion in this condition is the contracted and scarred pericardium that tightly grasps the chambers of the heart as if in a vise. The impeded diastolic filling of the heart causes an increased venous pressure with chronic passive congestion, ascites, and edema, even in the presence of a normal myocardium and competent valves. The operative relief of this condition constitutes one of the most dramatic chapters of modern surgery (1) (2).

Since the proper action of a heart of normal size can be restored by freeing it from a constricting pericardium, the suggestion has been made that the function of an hypertrophied and dilated heart might also be improved by removal of the structures that limit diastolic filling. As the normal pericardium stretches almost without limit in the face of a slowly developing cardiac enlargement, it is the unyielding bony framework of the chest that serves ultimately to restrict the greatly dilated or hypertrophied heart. Assuming at the outset that further dilatation of the heart will increase its functional capacity an effort may be made to give room for further dilatation by surgical removal of the overlying ribs and costal cartilages—so-called "decompression."

With these clinical problems in mind the action of the cat's heart has been embarrassed by imposing a partial obstruction to the outflow of blood. The function of the pericardium under such circumstances has been considered, with particular reference to the deleterious effects on the circulation that may result from the limitation of cardiac dilatation.

The experimental literature on the function of the pericardium is not extensive. Barnard (3) in 1898 reported to the Physiological Society certain studies on the pericardium and concluded that it was an inelastic structure preventing excessive dilatation of the heart.

Kuno (4) in 1915, using a Starling heart-lung preparation and an experimental method enabling him to open and close the pericardium at will, found that opening the pericardium caused a fall in venous pressure, a rise in arterial pressure and an increase in cardiac output. This effect was more marked at low venous pressures and with slow pulse rates. He also noted that hearts unsupported by the pericardium were apt to show hemorrhages beneath the epicardium and valvular insufficiency at high venous pressures. These effects were not observed with high venous pressures when the pericardium was intact. He concluded that "the existence of the pericardium is thus necessary for the unimpaired working of the heart in normal life."

Yamada (5) in 1917 confirmed these observations of Kuno on the heart-lung preparation. He also widely opened the pericardium in dogs, exercised them by running and examined the heart two to eight days after operation. He failed to find any evidence of dilatation or intramural hemorrhage and thought the absence of these conditions due to the rapid pulse rate occurring with exercise. The experiments in which he attempted to demonstrate the effects of rapid heart rates are not convincing because adrenalin was employed to increase the heart rate while its other physiologic effects were apparently not considered.

Wilson and Meek (6) in 1927 studied in roentgenograms the restraining influence of the pericardium on the size of the heart. After severing the diaphragmatic attachment of the pericardium in the dog they found that the pericardium began to exert a restraining influence on the diastolic size of the heart at a venous pressure of 0 cm of water and that at a venous pressure of 8 cm the heart completely filled the pericardium.

Van Liere and his collaborators (7) (8) in 1927 and 1930 produced acute cardiac dilatation by anoxemia and stimulation of the vagus nerve simultaneously recording the size of the heart shadow by roentgenograms. They found in the majority of their experiments a greater dilatation of the heart, after removal of the pericardium.

On the other hand, Felix (9) studying the influence of the pericardium upon hearts with artificially produced valvular defects, concluded that opening the pericardium had a favorable influence upon hearts with mitral or tricuspid lesions and suggested adopting this procedure in patients. In a recent article (10) he has published some further investigations on this question in both acute and survival experiments. In experimentally produced mitral insufficiency of several weeks duration he fails to note any circulatory improvement on opening the pericardium. It seems not unlikely that the findings in his acute experiments are due to the production of a certain degree of tamponade by the closure of the pericardium after creating the valvular defect. The reopening of the pericardium then improved cardiac action as a matter of course.

Moore (11) and Grant (12) reviewing the cases of congenital pericardial deficiency reported in the literature, conclude that the pericardium does not play an important part in supporting the wall of the heart. Experimental removal of the pericardium in animals is apparently compatible with active life (13).

In the following experiments we have studied (A), the effect of opening the pericardium upon the function of the normal heart as indicated by systemic and venous blood pressures, (B), the effect of opening the pericardium when the work of the heart has been greatly increased by a partial obstruction to the outflow of blood from one ventricle, (C), the effect of removing a constricting pericardium in the presence of a partial occlusion of the pulmonary artery or aorta, and (D), the tamponade effects produced by increasing the work of the heart within a snugly adjusted pericardium.

#### TECHNIC

The experiments were performed on cats anesthetized by the intraperitoneal injection of a 10 per cent solution of sodium barbital, 4.5 cc per kilogram of body weight. The injection was made one half to

three quarters of an hour before the experiment was started Access to the pericardium was obtained by removal of the lower two thirds of the sternum Both pleural cavities were widely opened and artificial respiration was maintained throughout the experiment by intermittent intratracheal insufflation The mean carotid pressure was recorded in the usual manner with a mercury manometer The venous tracing was obtained by the method described by Lewis (14) A long slender glass cannula was passed through the external jugular vein into the superior vena cava and connected with a saline manometer at the top of which was a small Brodie bellows recorder A solution of heparin was given intravenously to prevent clotting

These preliminary procedures were carried out in all the experiments described below It should be noted that this preparation entails the exposure of all the intrathoracic structures to atmospheric pressure with a consequent diminution in cardiac output (15) and presumably a slight decrease in diastolic heart size

#### RESULTS

*A Effect on the normal heart of opening the pericardium* The effect of opening the pericardium upon the venous and arterial pressures was noted in six experiments The pericardium was slit wide open from base to apex with a pair of fine scissors No change in venous or arterial pressure was observed in any of the experiments (fig 1) Occasionally there was a slight initial disturbance with a quick return to previous levels This disturbance could be reproduced by pressing the scissors upon the right ventricle or by drawing up on the pericardium with a pair of forceps, and was probably caused by such manipulation rather than by the actual opening of the pericardium

These observations fail to corroborate those made in heart-lung preparations by Kuno (4) and Yamada (5) who found a fall in venous pressure, a rise in arterial pressure and an increased cardiac output on opening the pericardium It should be recalled, however, that their observations were not made on the intact pericardium, but on one which had been previously opened and then drawn together again with sutures That such a method can very easily cause cardiac tamponade is shown below (C) Neither author records the effect upon the venous and arterial pressures of the initial opening of the intact

pericardium. The pulse rate in these six experiments varied from 136 to 224 per minute. There was no change in rate on opening the pericardium. The cardiac rate of the dog's heart used in heart-lung preparations is often only one half as rapid. According to the views of Yamada this may possibly account for some of the discrepancies between the results recorded here and those obtained by Kuno.

*B. Effect of opening the pericardium upon a heart with a partial obstruction to the outflow of blood from one ventricle.* The work performed by the heart is roughly the product of the cardiac output multiplied by the arterial resistance (16) (17). In these experiments the

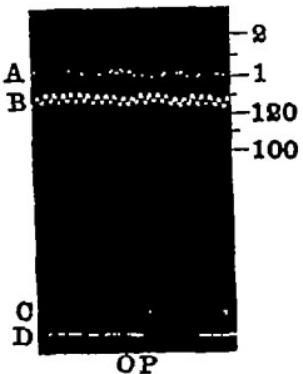


FIG. 1 KYMOGRAPHIC TRACING AT MOMENT OF OPENING PERICARDIAL CAVITY

A, venous pressure in centimeters of water recorded from the superior vena cava. B, arterial pressure in millimeters of mercury recorded from left carotid artery. C, time in 5 second intervals. D, signal marker. At OP the anterior surface of the pericardium was slit from base to apex.

work of the heart was increased by augmenting the arterial resistance. For this purpose a special clamp was devised for graduated compression of the pulmonary artery or aorta. The clamp is very similar to the one used by Haggart and Walker (18). However, it is capable of finer adjustments and can be employed in the "closed" chest. It consists of a tube A (fig. 2) to which is fixed the upper jaw of the clamp. The rod BC passes through the tube A and is bent to form the lower jaw of the clamp. The upper half of BC is wormed and articulates with the knurled nut D. An eighth of a turn of the nut moves the lower jaw of the clamp 0.079 mm.

Graduated compression of the pulmonary artery or aorta by this clamp was employed in these experiments only as a means of increasing the work of the heart by increasing the peripheral resistance. The circulatory disturbances resulting from such compression will be more fully described in another communication. It is interesting to note, however, that the sudden fall in arterial and rise in venous pressures

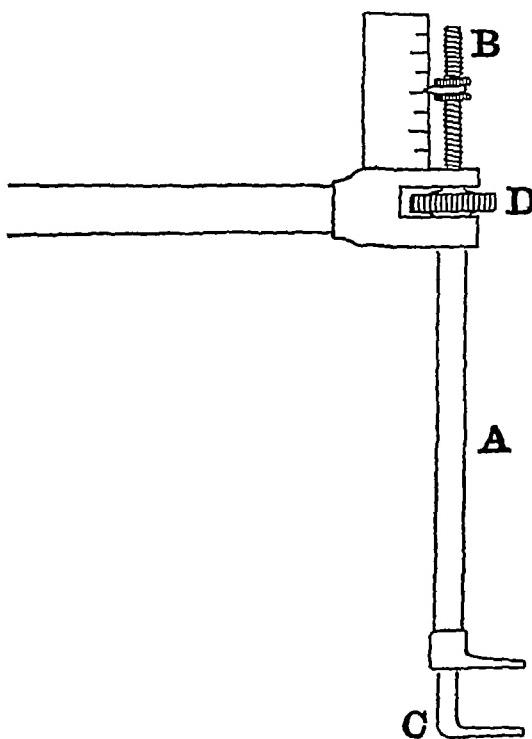


FIG. 2 CLAMP DESIGNED FOR GRADUATED COMPRESSION OF THE PULMONARY ARTERY OR AORTA  
(See text)

observed by other investigators, Cohnheim (19), Haggart and Walker (18) and Moore and Binger (20), during compression of the pulmonary artery or aorta have not been observed in our experiments when the compression was very gradually induced. There is on the contrary, a slow fall in arterial pressure and a corresponding rise in venous pressure. The kymographic record from Experiment 21 showing this

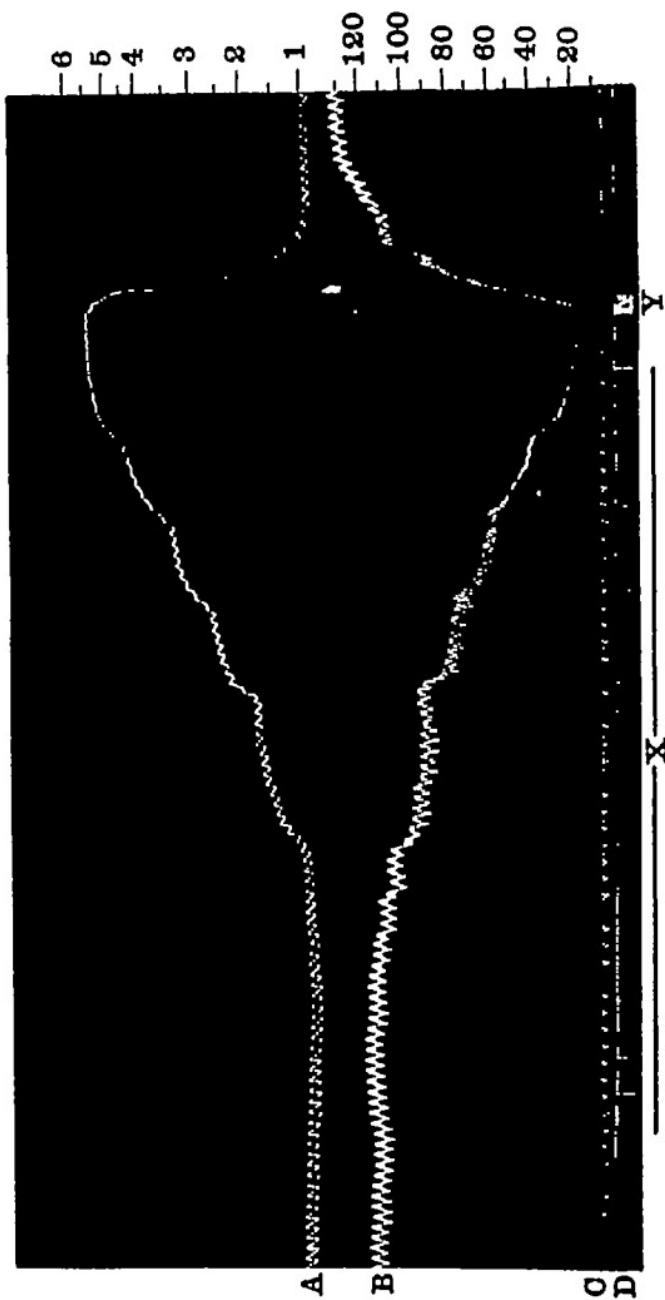


Fig. 3 KYMOGRAPHIC TRACING, EXPERIMENT 21  
 Venous and arterial pressures as in figure 1. During the time interval X, the clamp on the pulmonary artery was tightened by successive increments recorded by the signal marker. A slow fall in arterial and a rise in venous pressures resulted. At Y the clamp was completely released.

effect is reproduced in figure 3. In this experiment the anterior surface of the pericardium was incised from base to apex, completely freeing the heart. The clamp was then adjusted about the pulmonary artery just above the pulmonary valves. Very gradual compression of the artery with the clamp (0.079 mm at a time) produced a slow decline of arterial pressure and a slow rise in venous pressure. Figure 4 is a tracing from the same experiment a few minutes later, illustrating the effect of more abrupt compression. Here the successive incre-

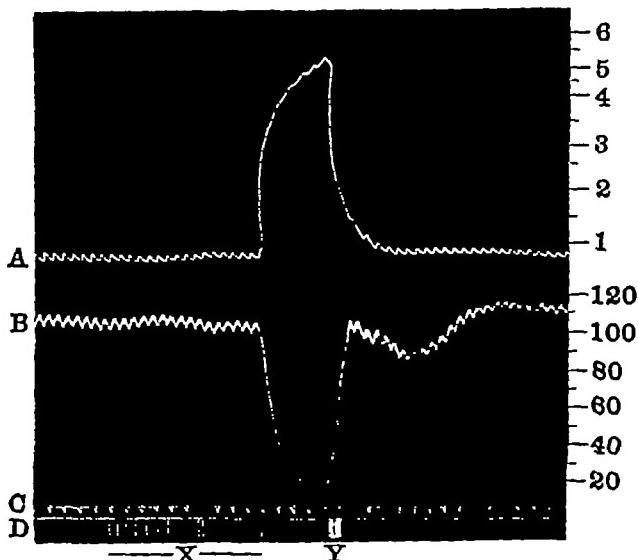


FIG. 4 KYMOGRAPHIC TRACING, EXPERIMENT 21

Venous and arterial pressures as in figure 1. Clamp tightened during time interval X and released at Y. Compression of the pulmonary artery in a more abrupt manner than shown in figure 3 produced a sudden failure of the heart.

ments of compression were larger (0.635 mm) and heart failure occurred abruptly as has been described by the above mentioned observers. The same results have also been observed during slow and rapid compression of the aorta.

Opening the pericardium after graduated compression of the pulmonary artery or aorta was carried out in ten experiments. In three of these the aorta was compressed and in seven the pulmonary artery. A small transverse incision, 1.5 cm long, was made in the base of the

pericardium directly overlying the pulmonary artery or aorta. The incision exposed only that portion of the vessel about which the clamp was to be placed and did not overlie the heart itself or presumably affect the normal relationship between the pericardium and the heart. The clamp was then placed in position and the artery compressed until there was a definite fall in arterial pressure and a rise in venous pressure. Opening the pericardium at this point by incising its anterior surface from base to apex was without effect on either venous or arterial pressures in five experiments. In one experiment the arterial pressure fell slightly and in another rose slightly. In two experiments the arterial pressure was progressively falling and the venous pressure rising when the pericardium was opened, the heart continued to fail following the procedure. Finally, in one experiment there was some improvement in the circulation on opening the pericardium (*OP*, fig 5), evidenced by a slight rise in the carotid pressure and a slight fall in superior caval pressure.

Under the conditions of these experiments the pericardium seems to offer no material support to a heart seriously decompensated by an obstruction to the outflow of blood. In no instance did removal of the pericardium in itself precipitate heart failure. In one instance (fig 5) the removal of the pericardium actually enabled the heart to overcome its handicap more effectively.

*C Effect of removing a constricting pericardium in the presence of an obstruction to the outflow of blood from one ventricle.* In order to study the functional relations of the pericardium to the heart under different experimental conditions, a method for opening and closing the pericardium was employed which is very similar to the method devised by Kuno. The anterior surface of the pericardium was incised longitudinally from base to apex. Three or four interrupted silk sutures were placed in the cut edges and drawn through holes in a small strip of celluloid (fig 6). By drawing these sutures up through the strip of celluloid and clamping them with a small artery clip the pericardium could be closed, and by releasing the threads, opened. If the threads were drawn up tightly and clamped, tamponade effects resulted (*CP*, fig 7). On releasing the threads, and thus opening the pericardium, the arterial and venous pressures returned to their for-

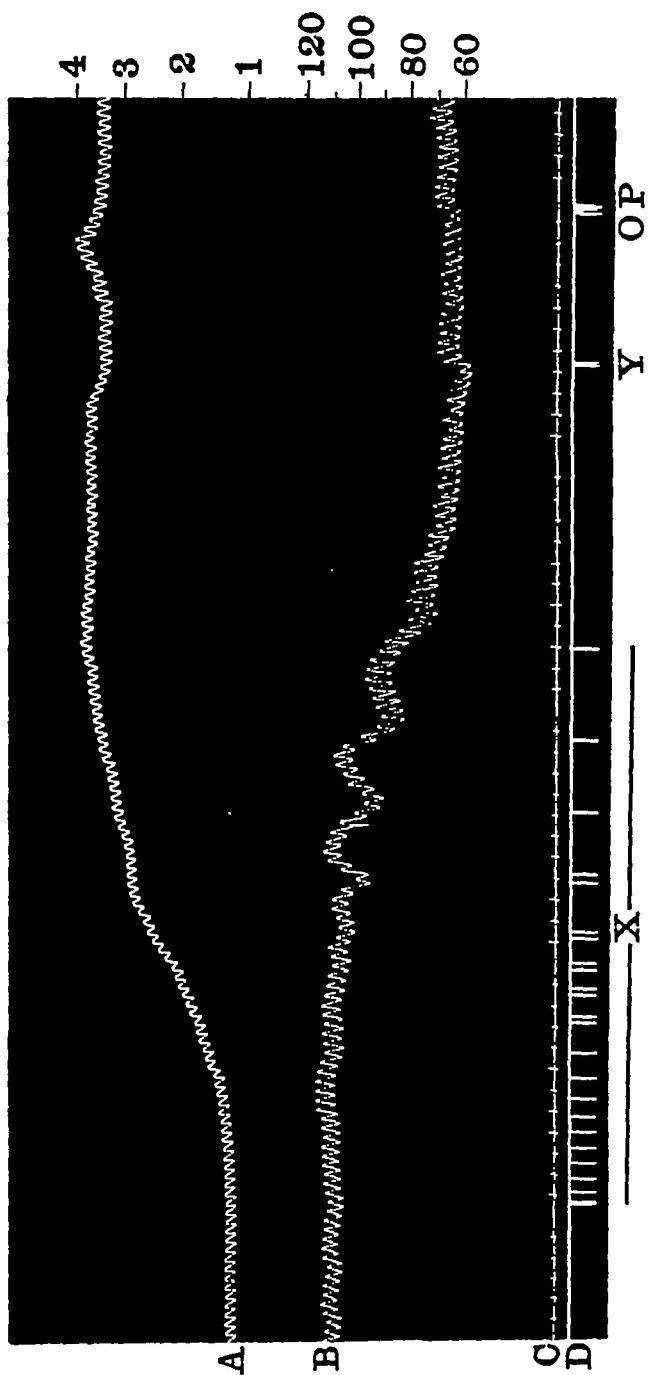


FIG. 5 Kymographic Tracing Illustrating the Opening of the Pericardium in the Presence of Partial Occlusion of the Pulmonary Artery

Venous and arterial pressures as in figure 1. Pulmonary artery gradually compressed during time interval X. Slight release of clamp at Y to prevent further cardiac failure. Opening of the pericardium (OP) gave but minimal circulatory improvement.

mer levels (*OP*, fig 7) This effect was recorded thirty-one times in thirteen experiments

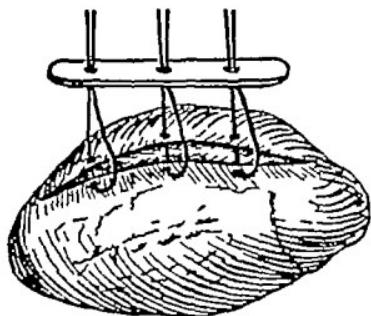


FIG 6 DIAGRAM ILLUSTRATING METHOD OF OPENING AND CLOSING PERICARDIUM

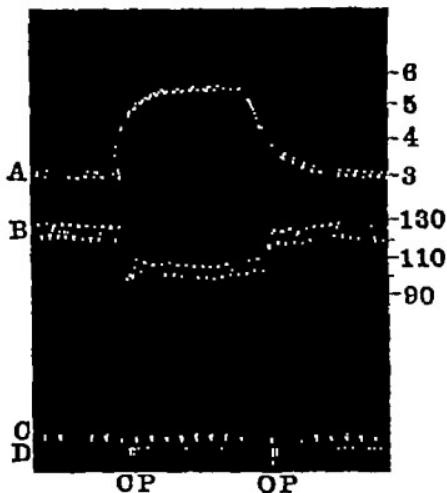


FIG 7 KYMOGRAPHIC TRACING SHOWING TAMPOONADE EFFECTS OBTAINED BY CLOSING PERICARDIUM AFTER METHOD OF KUNO

Arterial and venous pressures as in figure 1 At point *CP* the pericardium was closed and at *OP* opened

The cardiac tamponade so produced is similar to that occasioned by an experimentally produced pericardial effusion (19) (21). The extent of the deviations in arterial and venous pressure levels was

found to be dependent upon the degree of diminution in the original volume of the pericardium. The tighter the threads were drawn together, the more marked was the increase in venous pressure and the decline in arterial pressure. Releasing the threads and thus opening the pericardium, always resulted in a rise in arterial and a fall in venous pressures. It was then determined whether this effect is to be observed after greatly increasing the work of the heart by partial obstruction of the pulmonary artery or aorta and thus still further lowering the arterial and raising the venous pressures.

In Experiment 20 the pericardium was incised from base to apex. The clamp was placed about the pulmonary artery, and sutures for artificially opening and closing the pericardium were placed as described above. The threads were drawn up tightly, producing a slight cardiac tamponade (*CP*, fig 8). The pulmonary artery was compressed (*X*, fig 8), producing a further fall in arterial pressure and rise in venous pressure. Opening the pericardium by releasing the threads (*OP*, fig 8) produced a fall in venous, and a rise in arterial, pressure. This procedure was carried out seventeen times in nine experiments. In three experiments the aorta was compressed and in six the pulmonary artery. In every instance, except one, a similar rise in arterial and a fall in venous pressures occurred. In the one exception the heart was rapidly failing when the pericardium was opened, as evidenced by a constantly falling carotid pressure and rising venous pressure.

The rise in arterial and fall in venous pressures observed in these experiments evidently indicates an increase in cardiac output. As the resistance offered by the clamp to the outflow of blood is the same both before and after opening the pericardium, the increase in output indicates an increase in the work performed by the heart. Therefore these results apparently indicate that the heart can accomplish more work if the restraining influence of a constricting pericardium is removed.

The cardiac tamponade resulting from tight closure of the pericardium by sutures is obviously very similar to that existing in cases of *concreto pericardii*, where the normal return of venous blood to the heart is impeded by a rigid, thickened pericardium. Removal of such a pericardium results in circulatory improvement, as does opening of

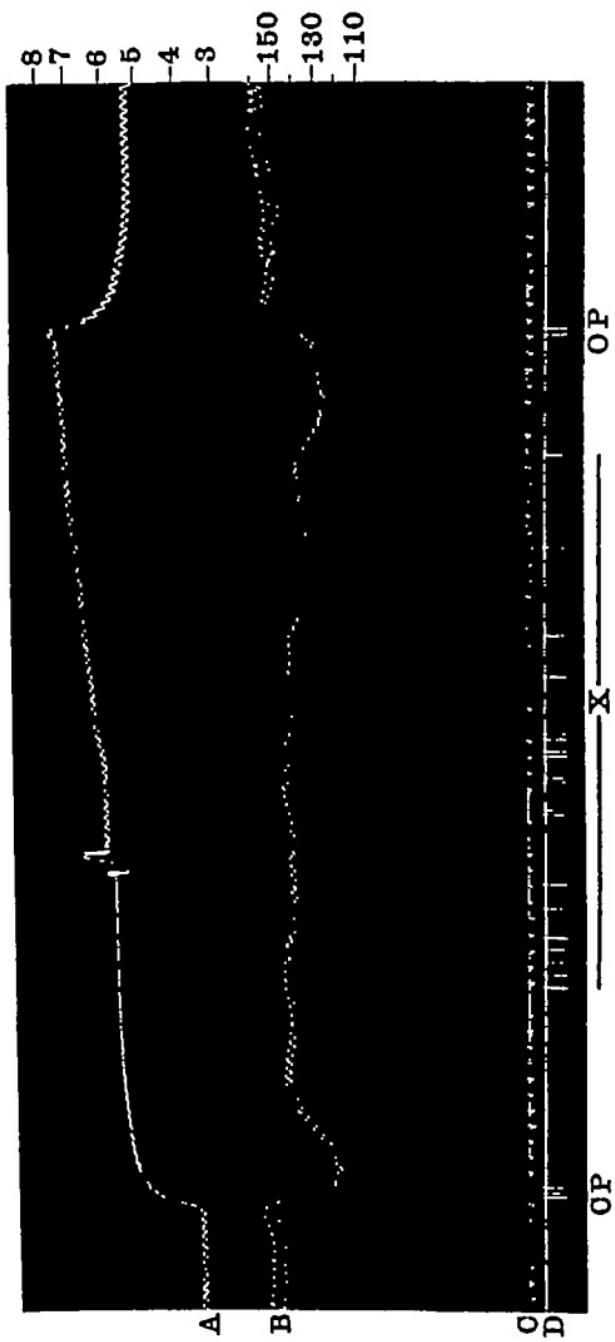


FIG. 8 KYMOGRAPHIC TRACING, EXPERIMENT 20  
Arterial and venous pressures as in figure 1. Pericardium was closed at point CP producing moderate tamponade. Clamp about pulmonary artery tightened during time interval X. Opening of pericardium at OP

the pericardium in these experiments. These results give no indication that the removal of a pericardium which is causing cardiac tamponade may be harmful in the presence of a normal heart muscle. This is true even though the work of the heart may be greatly increased by partial occlusion of the pulmonary artery or aorta.

*D Tamponade effects produced by increasing the work of the heart within a snugly adjusted pericardium and relieved by opening the pericardium.* It has been mentioned in the preceding section that closure of the pericardium by means of sutures resulted in cardiac tamponade.

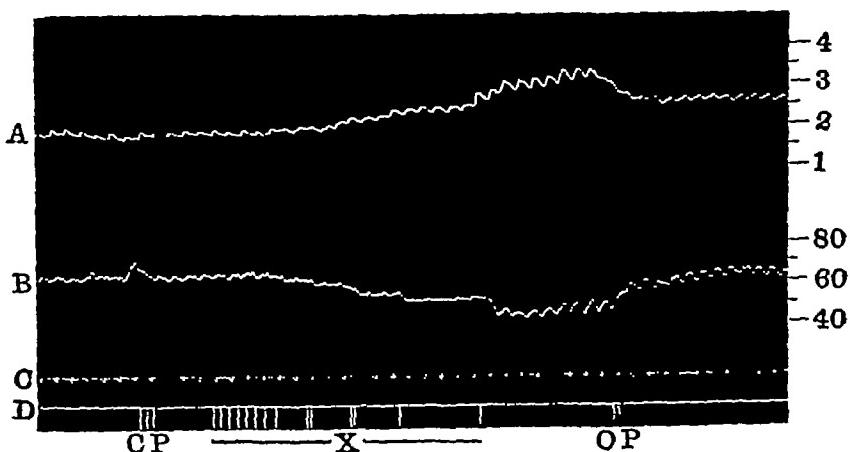


FIG 9 KYMOGRAPHIC TRACING, EXPERIMENT 17

Arterial and venous pressures as in figure 1. Pericardium closed at point *CP* to snugly envelop heart but without tamponade effects. Pulmonary artery compressed during time interval *X*. Opening of pericardium at *OP*.

However, it was found that by exerting great care in drawing the sutures up through the celluloid strip, the pericardium may be closed without producing tamponade. It was possible to draw the pericardium so snugly about the heart that the slightest additional tightening of the threads produced a fall in arterial, and a rise in venous, pressure. Opening the pericardium after such closure was without effect on venous or arterial pressure levels. The effect of opening such a pericardium on a heart whose work had been greatly increased by means of the clamp was determined in the following experiments.

In Experiment 17 the pericardium was incised longitudinally from base to apex. The clamp was placed about the pulmonary artery and sutures for artificially opening and closing the pericardium were placed as described above. The threads were drawn as tightly as possible without affecting either arterial or venous pressures (*CP*, fig 9) and clamped. The pericardium was thus drawn up snugly about the heart without producing the slightest degree of cardiac tamponade. The pulmonary artery was compressed until a well marked rise in venous pressure and fall in arterial pressure occurred (*X*, fig 9). When the pericardium was then opened by releasing the threads, a fall in venous, and a rise in arterial, pressure occurred (*OP*, fig 9).

This procedure was carried out eighteen times in seven experiments. In ten instances opening the pericardium caused a rise and fall in arterial and venous pressures respectively, similar to that shown in figure 9. In five instances the arterial pressure was unchanged but the venous pressure fell. In two instances no effect was observed in either venous or arterial pressures, and in one instance the heart was failing before the pericardium was opened and continued to fail thereafter.

The method of closing the pericardium employed here, namely drawing up on the sutures until any further tightening caused a rise in venous and a fall in arterial pressures, may be said to cause a diminution in the original volume of the pericardial cavity. Such a conclusion appears justified by a comparison of the results obtained on opening the pericardium in these experiments and in those described under section B. The relationship between the heart and pericardium in the experiments just described bears some similarity to that which exists between an enlarged heart and the structures that limit its diastolic expansion. In one case the pericardium has been made smaller, and in the other the heart has become larger. In both instances the difference between the diastolic heart size and the room available for expansion is diminished. These experiments then lend support to the observations already on record (9), that decompression of the heart may in favorable instances enable it to compensate more adequately for valvular defects.

## SUMMARY AND CONCLUSIONS

The following observations have been made on cats with open chests under artificial respiration

1 Opening the pericardium is without effect upon either arterial or venous pressures

2 Gradual compression of the pulmonary artery results in a slow fall in arterial pressure and a corresponding rise in venous pressure

3 Opening the pericardium when cardiac decompensation has been produced by compression of the pulmonary artery or aorta is without significant effect on the elevated venous pressure or the low arterial pressure

4 Tight closure of the pericardium with sutures produces cardiac tamponade with a fall in arterial and a rise in venous pressures On opening the pericardium again, the pressures return to their former level

5 When the pericardium has been closed with the production of cardiac tamponade and partial obstruction of the pulmonary artery superimposed, opening the pericardium causes a rise in the carotid pressure and a fall in the superior caval pressure

6 By exercising considerable care it is possible to close the pericardium with sutures without affecting either venous or arterial pressures Subsequent opening of the pericardium is similarly without effect on pressure levels

7 When the pericardium has been snugly closed by sutures without affecting either arterial or venous pressures and cardiac decompensation produced by compression of the pulmonary artery, opening the pericardium causes a rise in arterial pressure and a fall in venous pressure

8 In none of the twenty-eight experiments performed was the removal of the pericardium detrimental to the circulation, even when signs of cardiac decompensation had been experimentally produced

9 Increased work is accomplished by increase in the diastolic size of the heart Removal of a pericardium which limits this normal cardiac dilatation materially lessens the degree of cardiac decompensation

10 An analogy is drawn between these experimental observations

and the so-called "decompression" of enlarged hearts by removal of the bony framework of the precordial area

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# STUDIES ON COLLATERAL CIRCULATION I THERMIC CHANGES AFTER ARTERIAL LIGATION AND GANGLIONECTOMY

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(Received for publication February 26 1931)

The temperature of a part of the body depends largely upon its blood supply. Under certain conditions the temperature may therefore be a fair measure of the circulation. With this in view, a series of experiments was planned to approach the problem of collateral circulation and eventually the mechanism of vasomotor action. The group of experiments here presented deals with the thermic changes occurring in the hind feet of the dog (a) following ligation of the external iliac artery, and (b) following ligation of both external iliac arteries and unilateral removal of the tributary sympathetic ganglia.

## METHOD

The animal was given sodium amytal [Lilly] (50 to 75 mgm., i.e., 0.5 to 0.75 cc. of a 10 per cent solution per kilogram of body weight) intraperitoneally and was attached by thermopiles to a Leeds and Northrup potentiometer temperature recorder<sup>1</sup> about half an hour later. The four leads of the recorder were connected to register subcutaneous temperatures of the right foot (1), and of the left foot (2), temperatures of the rectum (3), and of the room (4) respectively, once every four minutes. After an equilibrium had been established, one or both external iliac arteries were exposed retroperitoneally and doubly ligated as close to the aorta as was practicable. In experiments (b) both external iliac arteries were similarly ligated and in one group of experiments the rami communicantes of the tributary sympathetic ganglia of one limb were severed and the ganglia removed after the temperature of both feet had decreased to room temperature and had remained there for some time; in another group the tributary sympa-

<sup>1</sup> The authors are indebted to the Leeds and Northrup Co., North Philadelphia, Pa. for the use of this instrument.

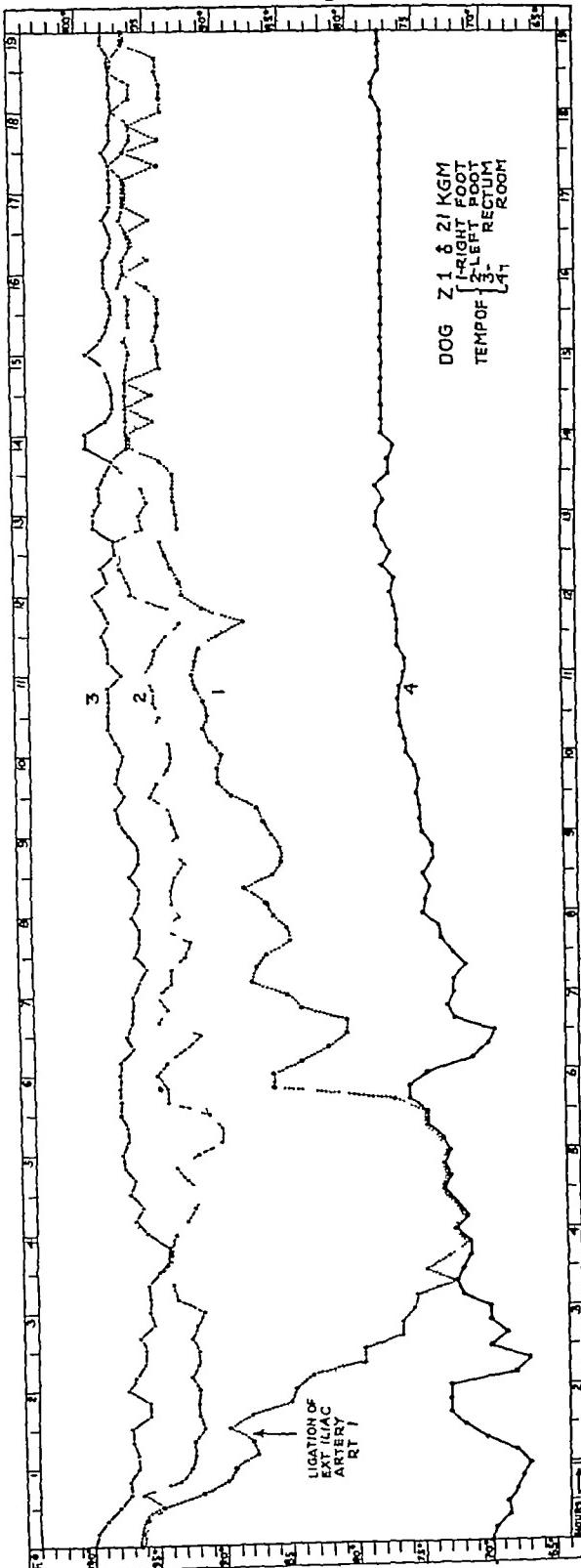


FIG. 1 DOUBLE LIGATION OF THE RIGHT EXTERNAL ILLIAC ARTERY CLOSE TO THE AORTA WAS FOLLOWED BY A DECREASE OF THE TEMPERATURE OF THE CORRESPONDING FOOT TO ROOM TEMPERATURE, A DROP OR APPROXIMATELY 20° F., WHERE IT REMAINED FOR NEARLY 2 HOURS. After numerous fluctuations with a trend upward, it reached the temperature of the other leg about the 13th hour following the ligation.

thetic ganglia were removed on one side immediately after ligation of the arteries

#### EXPERIMENTAL DATA

Ligation of the external iliac artery proximal to the profunda femoris was always followed by a marked decrease of the temperature of the foot of the corresponding limb. The lowering of the temperature was either gradual or sudden and usually the temperature of the foot reached that of the room in approximately 2 to 6 hours after a drop of about 10° to 30° F. In the majority of the experiments a rise in temperature and a return to the previous level occurred in about 13 hours following the ligation. The experiments were usually carried out on one limb, using the other as a control (fig. 1). The phenomenon, however, was approximately the same when both external iliac arteries were ligated, i.e., the decrease in temperature was simultaneous in both feet and the return to previous level also occurred at about the same time (fig. 2).

In those experiments in which both external iliac arteries were ligated and the temperature of both feet had decreased to room temperature, cutting of the rami communicantes and removal of the tributary sympathetic ganglia of one limb caused a sudden rise in temperature of the corresponding foot. The change in temperature of the foot from room temperature of about 70° F. to nearly that before ligation, usually occurred in about one hour and remained elevated until the end of the experiment several hours later, whereas the temperature of the other foot remained unchanged at room temperature (fig. 3). Transection of the rami communicantes and removal of the tributary sympathetic ganglia of one limb following ligation of its artery prevented the drop in temperature of the foot of the corresponding limb (fig. 4).

#### COMMENT

The reason for choosing the site of ligation as high as the origin of the external iliac artery from the aorta lies in the anatomic peculiarities of the blood supply of the lower extremities of the dog. The external iliac artery is a direct branch of the aorta in this animal and the profunda femoris leaves the external iliac artery within the pelvis. It was found that ligation of the femoral artery in Hunter's canal or of the external iliac artery distal to the origin of the profunda femoris led

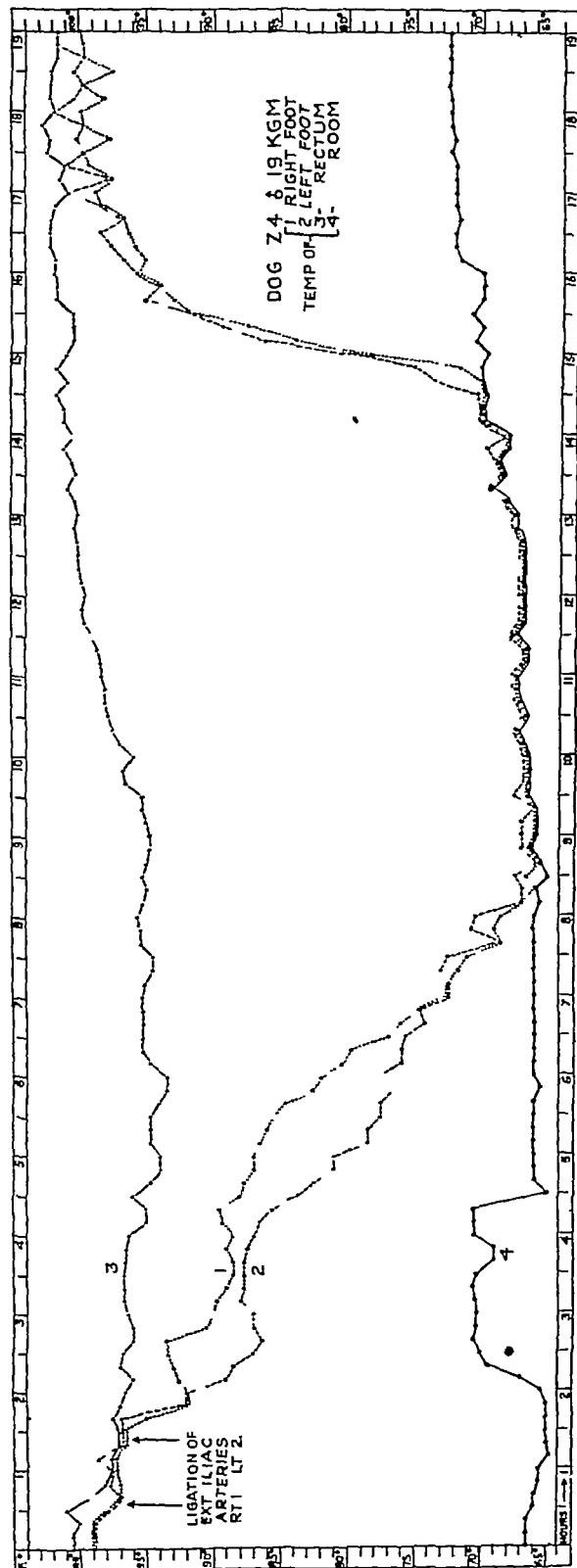


FIG. 2 LIGATION of Both EXTERNAL ILLAC ARTERIES Close to the Aorta Was FOLLOWED BY A GRADUAL DECREASE OF THE TEMPERATURE of Both Feet or NEARLY 30° F to ROOM TEMPERATURE It remained there for almost 6 hours, then with a steady rise it returned to the level before ligation

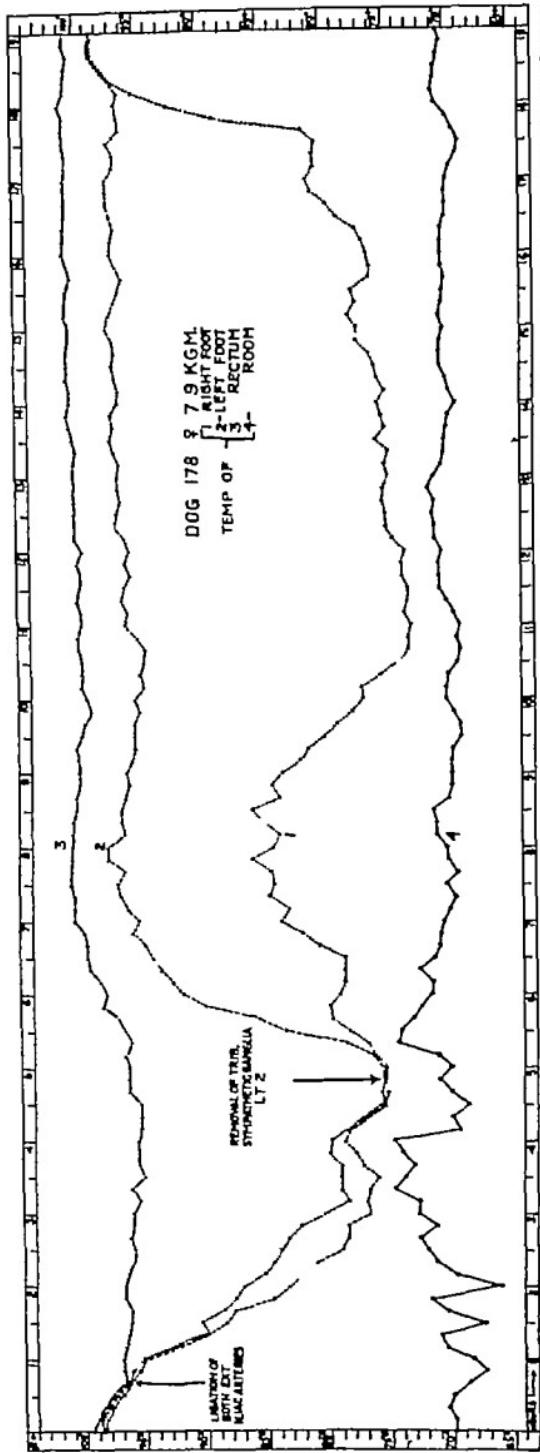


FIG. 3 DOUBLE LIGATION OF BOTH EXTERNAL ILLIAC ARTERIES CLOSE TO THE AORTA WAS FOLLOWED BY A DECREASE OF ABOUT 20° F. IN THE TEMPERATURE OF BOTH FEET. Transection of the rami communicantes and removal of the tributary sympathetic ganglia of the left limb caused a rise in temperature of about 20° F. of the corresponding foot. This temperature difference was maintained for about 12 hours when a rise in temperature of the other foot abolished the difference.

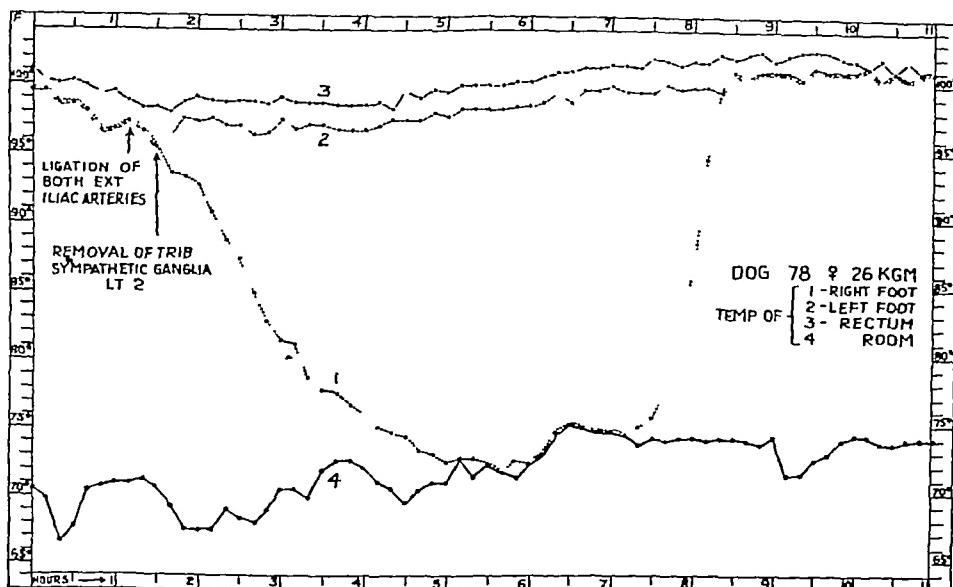


FIG 4 DOUBLE LIGATION OF BOTH EXTERNAL ILIAC ARTERIES CLOSE TO THE AORTA WAS FOLLOWED BY AN INITIAL DECREASE IN TEMPERATURE IN BOTH FEET Transection of the rami communicantes and removal of the tributary sympathetic ganglia of the left limb prevented a further drop in temperature in the corresponding foot while that of the other fell about  $25^{\circ}$  F The temperature of the right foot returned to the previous level about 6 hours after ligation

to no, or only slight and transient, change in temperature of the foot of the corresponding limb (1)

The above experiments show that after ligation of the external iliac artery the circulation in the corresponding limb is insufficient to maintain its normal temperature The circulation, however, as indicated by the temperature rise of the limb to the level of that before ligation, returns in about 13 hours This seems to indicate the presence of anatomical channels which are sufficient to care for the circulation after ligation and suggests that the reestablishment of former conditions by collateral circulation is a vasomotor phenomenon

This is further substantiated by the fact that the temperature of a limb which has dropped to room temperature after ligation of its external iliac artery can at any time be raised to the normal level by removal of the tributary sympathetic ganglia Not only that, but removal of the tributary sympathetic ganglia simultaneously with

ligation of the artery prevents the lowering of the temperature of that limb

#### SUMMARY AND CONCLUSIONS

In the dog ligation of one or both external iliac arteries close to the aorta was always followed by a decrease of temperature in the corresponding foot varying from about 10° to 30° F. In the majority of the experiments a rise in temperature with a return to its previous level occurred about 13 hours after ligation. This, in the writers opinion seems to indicate the presence of anatomical channels sufficient to care for the circulation after ligation and suggests that the reestablishment of former conditions by collateral circulation is a vasomotor phenomenon.

This is further substantiated by the fact that the temperature of a limb which had dropped to room temperature after ligation of its external iliac artery rose to normal soon after removal of the tributary sympathetic ganglia and that simultaneous removal of the tributary sympathetic ganglia with ligation of the artery prevented the lowering of the temperature of that limb.

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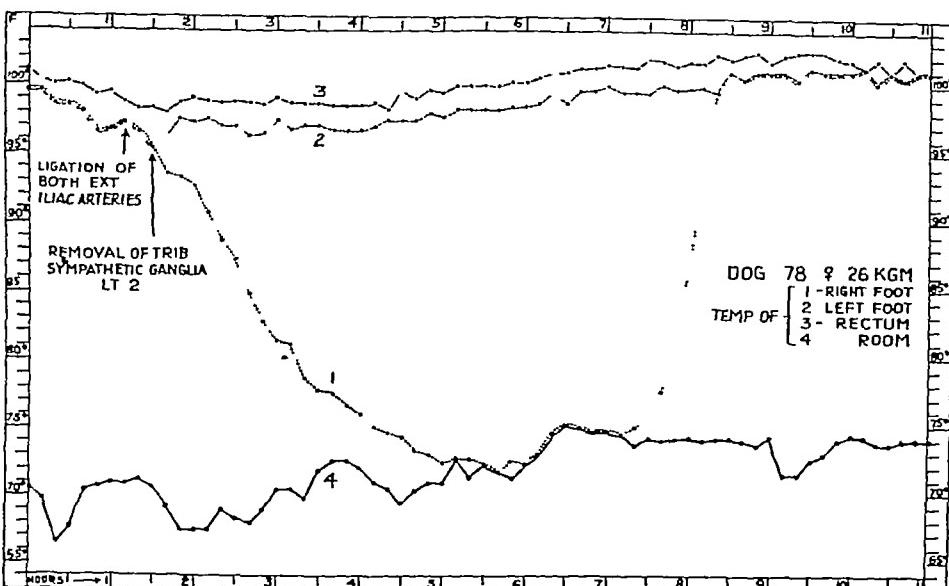


FIG 4 DOUBLE LIGATION OF BOTH EXTERNAL ILIAC ARTERIES CLOSE TO THE AORTA WAS FOLLOWED BY AN INITIAL DECREASE IN TEMPERATURE IN BOTH FEET Transection of the rami communicantes and removal of the tributary sympathetic ganglia of the left limb prevented a further drop in temperature in the corresponding foot while that of the other fell about 25° F The temperature of the right foot returned to the previous level about 6 hours after ligation

to no, or only slight and transient, change in temperature of the foot of the corresponding limb (1)

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This is further substantiated by the fact that the temperature of a limb which has dropped to room temperature after ligation of its external iliac artery can at any time be raised to the normal level by removal of the tributary sympathetic ganglia. Not only that, but removal of the tributary sympathetic ganglia simultaneously with

STUDIES ON COLLATERAL CIRCULATION II THERMIC  
CHANGES AFTER ARTERIAL LIGATION, SECTION  
OF SPINAL CORD OR POSTERIOR ROOTS  
AND GANGLIONECTOMY

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(Received for publication February 26 1931)

Previous experiments (1) have shown that in the dog ligation of both external iliac arteries close to their origin caused a decrease in the temperature of both hind feet to room temperature and that cutting of the rami communicantes and removal of the tributary sympathetic ganglia caused a rise in temperature of the corresponding foot to about that before ligation. The present experiments were planned to ascertain (a) whether division of the spinal cord at about the level of the first lumbar vertebra, and (b) section of the posterior roots below this level would influence the above reaction.

METHOD

As in the previous experiments, the animal was given sodium amyral [Lilly] intraperitoneally and attached to the Leeds and Northrup temperature recorder by thermopiles about half an hour later. After an equilibrium had been established, both external iliac arteries were exposed and ligated as in the previous experiments. In experiments (a) after the temperature of both feet had decreased to about room temperature and had remained there for several hours, the spinal cord was sectioned after laminectomy at about the level of the first lumbar vertebra. After sufficient time had elapsed for a reaction to appear, were it going to do so, the rami communicantes of the tributary sympathetic ganglia of one limb were severed and the ganglia were removed. In experiments (b) when the temperature of both feet had decreased to about room temperature after ligation of both external iliac arteries and had remained there for several hours,



Transection of all posterior roots below the level of the first lumbar vertebra on one side exerted no influence whatsoever upon the temperature of the foot which had dropped to room temperature after ligation of its external iliac artery. Subsequent transection of the rami communicantes and removal of the tributary sympathetic ganglia were promptly followed by increase in temperature of the corresponding foot from room temperature to nearly that of the foot before ligation.

Figures 1 and 2 illustrate the phenomena described.

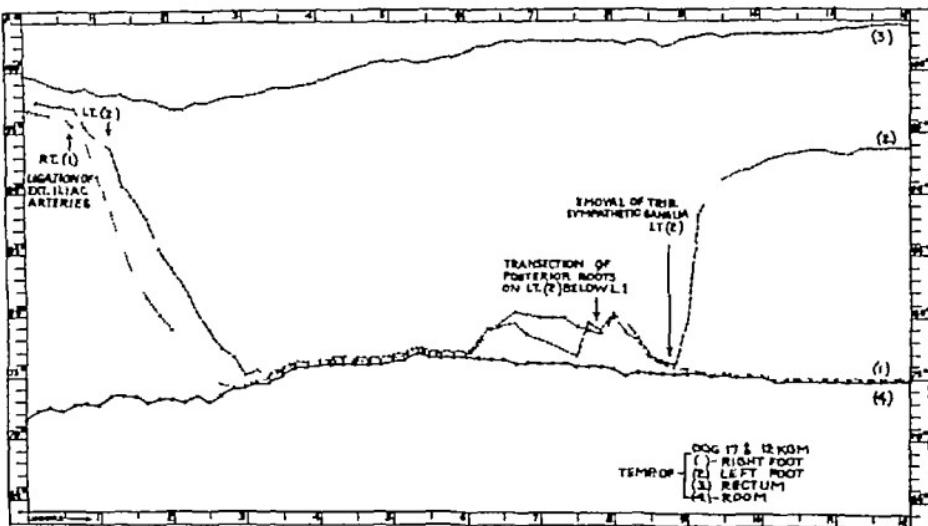


FIG. 2 FOLLOWING LIGATION OF BOTH EXTERNAL ILIAC ARTERIES THE TEMPERATURE OF BOTH FEET DROPPED 20° F. Transection of all posterior roots on one side below the level of the first lumbar vertebra had no influence whatsoever upon the lowered temperature of the foot. Subsequent transection of the rami communicantes and removal of the tributary sympathetic ganglia caused a prompt rise in temperature of the corresponding foot of 19° F i.e. from room temperature to nearly that of the foot before ligation.

#### COMMENT

Under the above experimental conditions transection of the spinal cord at the level of the first lumbar vertebra has no effect whatsoever upon the lowered temperature of the feet produced by ligation of both external iliac arteries. Subsequent removal of the tributary sympathetic ganglia, on the other hand, is followed by a prompt rise in

temperature of the corresponding limb from room temperature to nearly that of the foot before ligation. This occurred in exactly the same way as in the previous experiments in which the cord was not transected. These observations suggest that in these experiments bulbar impulses play no appreciable part in the mechanism of the development of collateral circulation.—Transection of all posterior roots below the level of the first lumbar vertebra on one side had no effect whatsoever upon the lowered temperature of the feet produced by ligation of both external iliac arteries. Removal of the tributary sympathetic ganglia of the corresponding limb was followed by a prompt rise in temperature of that limb in much the same manner as in those experiments in which the posterior roots had not been transected. These observations suggest that impulses communicating through the posterior roots below the level of the first lumbar vertebra play no appreciable part in the mechanism of the reestablishment of former conditions in the limb by collateral circulation, as observed in these experiments.

#### SUMMARY AND CONCLUSIONS

In the dog the lowered temperature of the feet produced by ligation of both external iliac arteries was not influenced by either (a) transection of the spinal cord at the level of the first lumbar vertebra or (b) transection of all the posterior roots on one side below the level of the first lumbar vertebra. Removal of the tributary sympathetic ganglia caused a prompt rise in temperature of the corresponding foot from room temperature to nearly that of the limb before ligation whether or not the spinal cord had been transected at the level of the first lumbar vertebra, or whether or not all posterior roots below this level on the corresponding side had been transected.

These observations suggest that (a) bulbar impulses and (b) impulses communicating through the posterior roots below the level of the first lumbar vertebra play no appreciable part in the mechanism of the development of collateral circulation, as observed in these experiments.

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# EXPERIMENTAL REFERRED PAIN FROM THE GASTRO-INTESTINAL TRACT PART I THE ESOPHAGUS

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(Received for publication March 30 1931)

Despite the development of modern diagnostic methods, the exact recognition of the disorders or lesions which are responsible for digestive symptoms remains a difficult problem. In practice the percentage of error in this domain of medicine is high and in many instances diagnosis is based more on the general clinical intuition of the examiner than on any very substantial basis. The interpretation of symptoms is especially difficult and it is with this phase of the subject that the present studies are concerned.

It is generally conceded that the first step in the analysis of a digestive disorder is a careful consideration of the patient's history, and symptom complexes have been built up which are believed by some to indicate with considerable certainty the existence of special lesions. Many physicians for example are quite dogmatic as to the significance of so called "peptic ulcer indigestion" or "gallbladder indigestion"; some go even further and feel that such conditions as "duodenitis," "mobile cecum," etc., present themselves with characteristic complaints. The validity of such symptom complexes, however, still remains open to question and our own experience indicates that unless fortified by some highly specific phenomenon such as bleeding in the case of ulcer or colic or jaundice in the case of gallbladder disease, the digestive symptoms in themselves (gas, vague abdominal distress, burning or "indigestion") have little, if any, specific meaning. When one considers that the upper abdominal viscera have a common autonomic innervation and that their sensibility under ordinary conditions is of a crude protopathic type this conclusion is supported. Furthermore, when one interrogates patients with digestive dis-

comforts and in response to the question is the pain dull, knife-like, radiating, cramp-like, diffuse or localized, almost always receives an equivocal answer it becomes evident that efforts to draw exact conclusions from minute differences in types of discomfort rest on an unsound basis

To this statement exception must be made with reference to two clearly defined and intelligible types of pain—severe hollow viscous colic whether it be of renal, biliary or intestinal origin clearly indicates forcible contraction of the structure in question in response to obstructive or inflammatory spasm, and intense localized pain with tenderness on pressure with almost equal certainty points to an actual lesion of peritoneum. But these two types of sensation which are of great diagnostic value occur in only a negligible minority of the great run of patients with digestive symptoms and they do not bear directly, therefore, on the point now at issue. At any rate it is clear that the problem of the interpretation of digestive symptoms still requires solution, and as it has been impossible by means of clinical observation to settle the question an experimental approach is desirable. This seemed feasible at least insofar as one might stimulate artificially various parts of the digestive tract and observe the character and location of such sensations as were produced with reference to features of practical diagnostic value.

Our plan, in brief, was to produce pain by inflating balloons at various levels of the gastro-intestinal tract and to examine a sufficiently large series of people so that statistical conclusions could be drawn as to site of reference of pain, its character and the relation of artificially produced pain to spontaneous pain in patients with digestive symptoms. The present paper concerns itself with studies of the esophagus only, the stomach, duodenum and colon will be discussed in a later communication.

#### LITERATURE

It is a matter of common experience that under thoroughly normal conditions the activities of the gastro-intestinal tract are carried on with a minimum of conscious sensation. Aside from the vague discomfort of hunger and the indefinite feeling of "fullness" after eating little is to be felt and indeed even these sensations are bound up with a wider somatic reaction which is clearly not directly referable to the stomach. More purposeful attempts to analyze the sensibility of the gastro-intestinal tract

in the main furnish confirmation of the essential silence of its operations as far as consciousness is concerned and the reader may be referred to the painstaking observations of Mackenzie (1) Hertz (2) and others and later of Carlson, Carlson and Braafadt (3) in experiments in which the exact nature of the procedure was unknown to the subject observed that the normal stomach mucosa was insensitive to tactile stimuli, that there was a crude (protopathic) reaction to the extremes of heat ( $45^{\circ}$  C) and cold ( $12^{\circ}$  C) and that stimulation of the mucosa increased reflex excitability of the spinal cord and induced changes in vasomotor tonus. Hertz among other things pointed out that while the stomach was ordinarily insensible to heat cold and the introduction of acid the esophagus reacted to these stimuli with sensations referred to the lower sternal region and epigastrium. Such purposeful observations have been amplified from day to day in the clinic by surgeons who cut and handle the gastro-intestinal mucosa directly at operation or through the proctoscope.

As far as the present mode of attack is concerned but little has been done previously. Payne and Poulton (4) attempted to analyze the motility of the esophagus in relation to spontaneous pain in clinical cases by registering contractions transmitted through a water filled balloon. They showed among other things that the sensation of 'heartburn' was associated with peristaltic movements of the esophagus. They subsequently elaborated their work (5, 6) and produced evidence that referred pain from the esophagus is caused by stretching and deformity of the pain endings in the wall. Nausea was produced by distension of the esophagus as well as referred pain but they report no systematic studies on relation of the site of stimulation and the situation of the referred pain. Hertz (2) inflated the esophagus in two normal men by means of a thin rubber bag. There resulted a sensation of fullness felt as if it were produced in the middle line deeply beneath the anterior surface of the body and the level of the stimulus was always accurately recognized. C. M. Jones (7) has also carried out somewhat similar experiments but no really extensive data are available.

#### APPARATUS AND METHODS

The mercury weighted stomach tube described by Wilkins was used. A small balloon was constructed around the perforations proximal to the mercury chamber. Rubber from an ordinary toy balloon was found satisfactory and durable. The balloon was made in such a way that when collapsed its walls approximated those of the stomach tube and when distended with 30 cc. of air it assumed an almost spherical shape with a diameter of about 3 cm. The tube was marked at intervals so that the position of the bag in the esophagus could be readily estimated and it was attached by a T connection on the one hand to a U mercury manometer and on the other to a luer syringe. (See Fig. 1.) The procedure consisted

of inflating the bag in the esophagus at various levels. We usually began at 20 cm from the teeth and advanced by intervals of 5 cm until the cardia was reached. Air was introduced by means of a 30 cc luer syringe at the rate of 2 cc per second (metronome) and the subject was requested to signal as soon as he felt anything, and to indicate, by pointing, the site of the sensation. The amount of air in the bag and the pressure were recorded at this point as well as the character of the sensation.



FIG 1 BALLOON USED IN ESOPHAGUS, COLLAPSED AND INFLATED

The following points were studied (1) The minimum amount of inflation (cc of air and pressure) necessary to produce sensation, (2) the site of the sensation (3) the character of the sensation, (4) the relation of site of sensation to position of balloon, (5) the relation of sensation to spontaneous clinical symptoms, and (6) the mechanism of the referred pain.

A miscellaneous group of ward patients were used some had digestive symptoms, but for the most part they were free of gastro-intestinal trouble. The object of the experiment was explained and the patients cooperated usually very intelligently.

#### THE SITE OF THE SENSATION

It soon became evident that there were sites of predilection for referred sensation *regardless of the position of the bag* (see below). These sites were at the lower end of the sternum and in the neck just

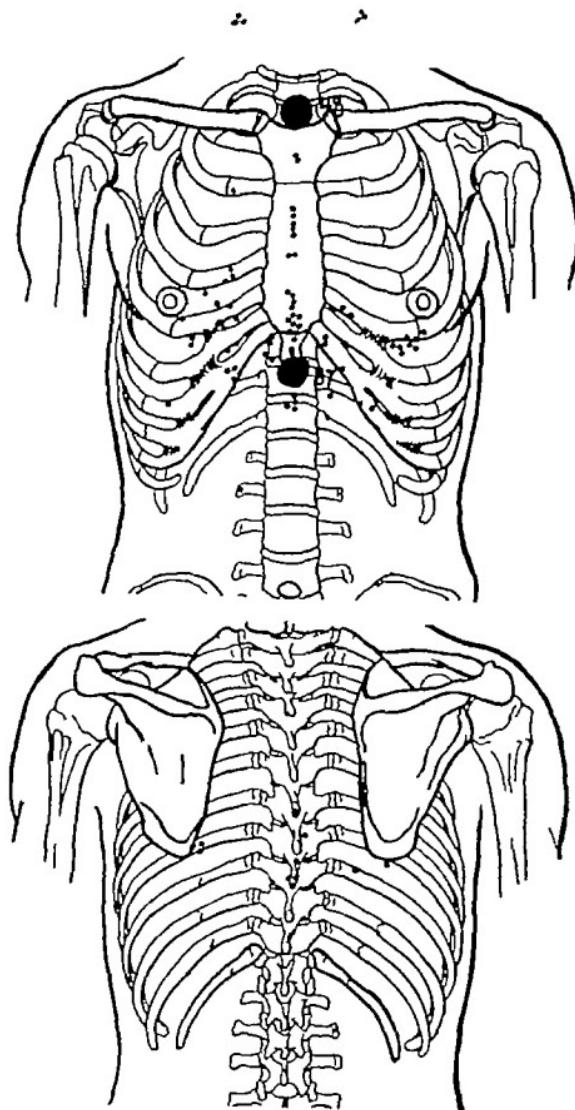


FIG 2 SITES OF REFERRED PAIN FROM INFLATION OF ESOPHAGUS  
Each dot indicates response to one inflation except the large circles which represent groups of 48 and 87 dots which fell too close for separate charting

TABLE I  
*Summary of site of referred pain from inflation of esophagus at various levels in each case. The small number of observations in the last column is due to the fact that the balloon had passed into the stomach at this level.*

Case Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Cm from teeth																			
20	1*	1	1	5	4R	2	1	2	1	5	1, 2	2L	1	2R	4	2R	2	1	
25	1	1	1	2	4R, 7R	2L	2	2	1	5	1, 2	2L	1	2R	3	2	2	1, 2	
30	5	1	4	6L	2, 7	2L	2R	2	2	1, 4	5R, L	1, 2	1, 2I	1	2	5	2	2	
35	1, 6	1	1	6	1, 2R	2L	1, 2R	1	1	5R, L	1, 2	1, 2L	1	2	2	2	1	2	
36-40	1, 6	1								1	2			1, 2					9

Case Number	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
Cm from teeth																					
20	1	3	2				1	4	3	2	4R	2R, L	3	2	4R	1, 2	2	2	2		
25	5R, L	3	4	2	2	8	4	5	5	2	4R	2R, L	5	1, 2	7L	1, 2	2	2	5		
30	5R, L, 7	4	2	2	2	8, 3, 4, 5	2	2L, 7	4	2	2, 9, 7	4R	2R, L	2	1, 2	7L, 5R, L	1, 2	2	2	2R, 6L, 3	
35	2L	2	2	2	2	1	2L, 7	5	2	4R	2R, L	6	2	7L, 5R, L, 1	1, 2	2	2	4			
36-40	2	6	1, 2				1					2	1, 2								

\* 1 = suprasternal notch

2 = xyphond

3 = upper sternum

4 = midsternum

5 = lower sternum

6 = epigastrium

7 = back

8 = neck

9 = face

R = Right of mid-line

L = Left of mid-line

above the suprasternal notch. As a rule the subject pointed definitely to one or another of these regions. However, other situations at various levels of the sternum to the left or right and in back were frequently indicated. Sometimes the subject pointed to two perhaps widely separated areas or first to one spot and then to another. It was evident that conditions of contraction or relaxation of the esophagus quite apart from the artificial stimulus might modify the referred sensation. This was brought out very clearly in many cases in which a repetition of the test after an interval of a few minutes yielded quite different results from the first experiment. Figure 2 shows graphically the total area implicated by all our experiments and Table I summarizes the findings in each case. The symbols indicate roughly the points at which pain was felt although it is not possible to bring out by this method the size of the area of referred sensation. R and L indicate that the pain spread from the mid line either to right or left or that it was located entirely to right or left. Table II shows very clearly the

TABLE II

*Summary of location of referred pain from inflation of esophagus at various levels*

Level of balloon from teeth	Location of pain (Number of times)								
	1	2	3	4	5	6	7	8	9*
cm									
20	12	17	3	5	1	0	0	0	0
25	9	19	2	4	6	0	2	0	0
30	7	24	2	5	5	2	5	1	1
35	12	19	0	2	3	3	2	0	0
40	8	8	0	0	0	2	0	0	0
Total	48	87	7	16	15	7	9	1	1

\* The numbers 1-9 have same meaning as in Table I

preponderance of suprasternal notch and xiphoid region as sites of pain these two areas being implicated 135 times in 191 inflations. Next in frequency come other areas behind the sternum with epigastrium, back, face and neck only occasionally affected. As pointed out above the sensation was usually vague and more or less widespread with a central point of maximum intensity. The diagrams must be

interpreted with this in mind as it was impossible to outline accurately the exact and complete distribution of the referred sensations

#### RELATION OF SITE OF SENSATION TO POSITION OF BALLOON

As pointed out above (see review of literature) some observers have concluded that pain from distension of the esophagus is referred to the body surface (sternum) at the level of the stimulus. Our observations were quite at variance with any such idea. This is clearly shown by Tables I and II and indeed there was only one case (Case 21) in which there appeared to be a constant relation between the site of the balloon and the referred sensation. The situation can best be clarified by diagrams of illustrative cases (Figures 3 to 9). It appears that with the balloon high in the esophagus pain may be referred to the xiphoid and vice versa, or that various permutations and combinations may occur. The occasional cases in which pain was referred to the face and high in the neck are of special interest, and also those instances in which the sensation lay definitely to one side of the mid-line. A possible explanation of this phenomenon will be discussed below, but the point of practical importance is that the site of pain does not indicate any definite site for the location of the stimulus.

The sensations so far described have been those which resulted from minimal stimuli, as soon as the subject felt anything the inflation was stopped and the pain was recorded. However, if further inflation was carried out the sensation might not only be accentuated at the original site but often spread widely or appeared in new situations. For example pain felt at first in the suprasternal notch on increased pressure spread up and down entire sternum and finally localized at the xiphoid, or, pain felt first 5 cm above xiphoid on increased pressure radiated to both shoulder blades. It is evident therefore, that no absolute effects can be expected but that endless variations will exist depending upon the intensity of the stimulus and the sensitiveness of the pain producing mechanism.

Even with stimuli as nearly as possible constant, repeated experiments with the same subject often gave different results. Figures 10 and 11, for example, show the results of inflations at intervals of a few minutes in Case 14. In other cases pain appeared first in one situation but if the balloon was left in situ without further inflation sensations developed in other perhaps widely separate regions.

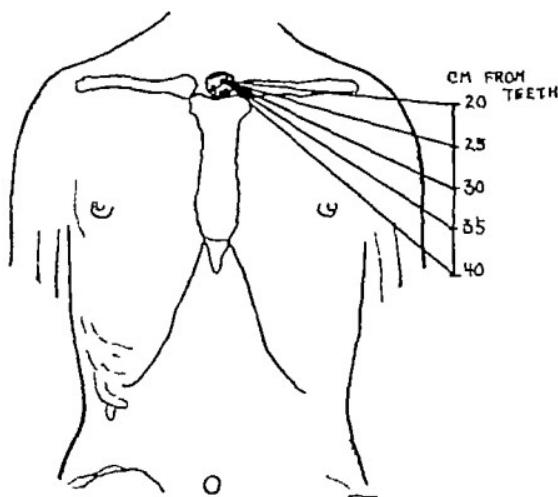


FIG 3 (CASE 2) IN THIS AS IN THE FOLLOWING DIAGRAMS THE FIGURES IN THE MARGIN INDICATE THE LEVEL OF THE BALLOON THE LINES RUN TO THE AREA OF REFERRED PAIN

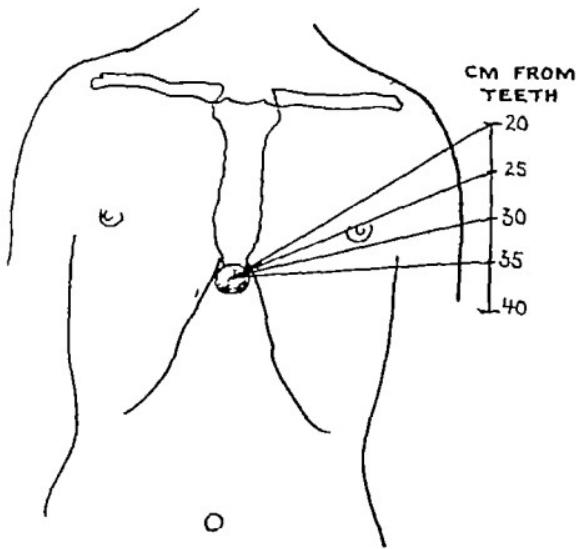


FIG 4 (CASE 36)

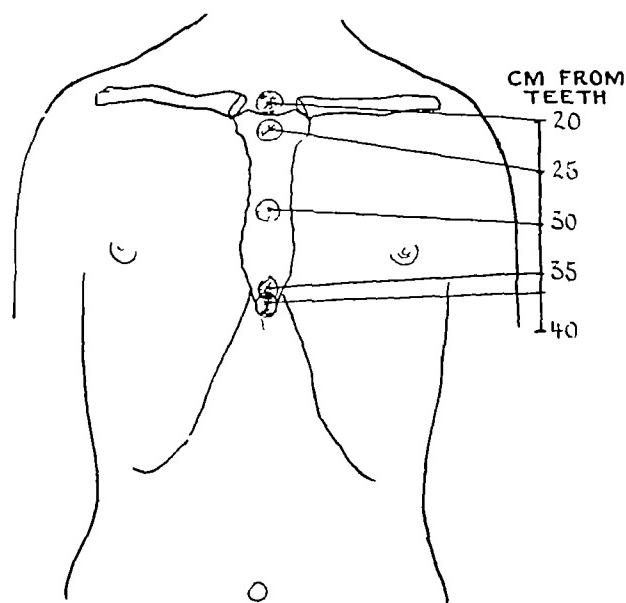


FIG 5 (CASE 21)

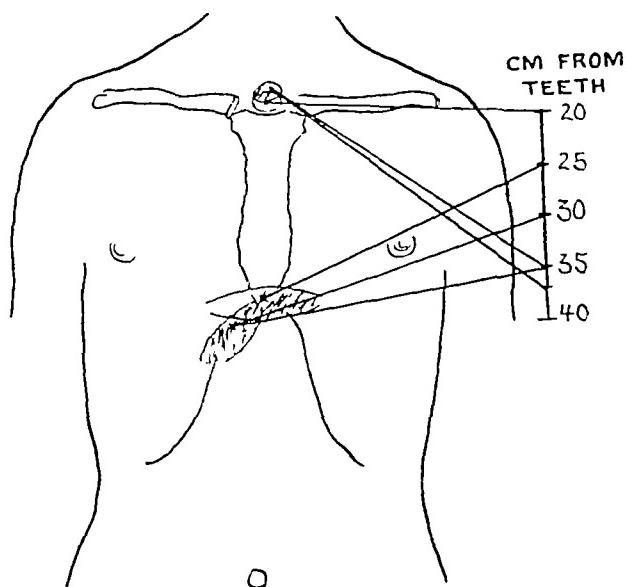


FIG 6 (CASE 7)

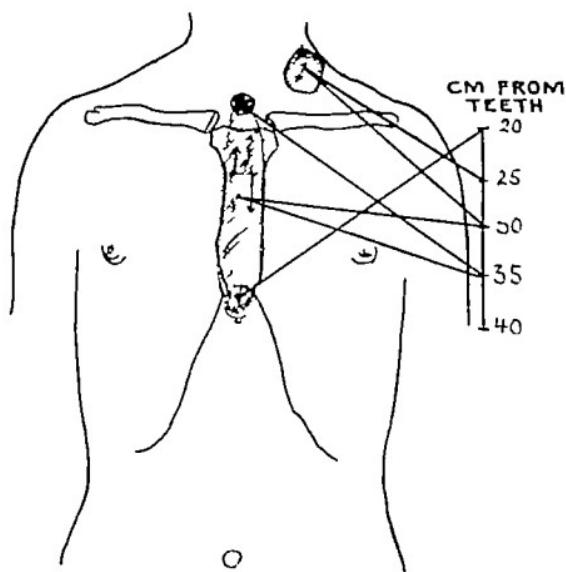


FIG 7 (CASE 25)

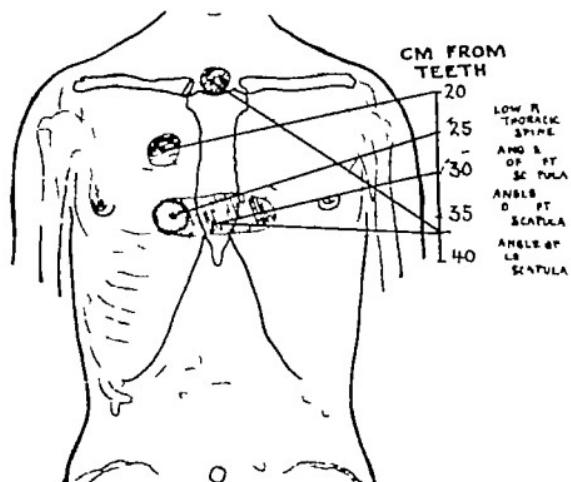
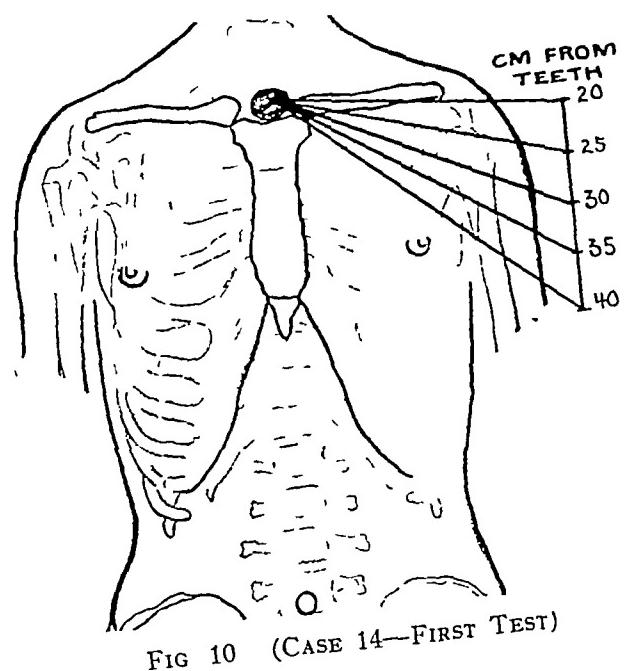
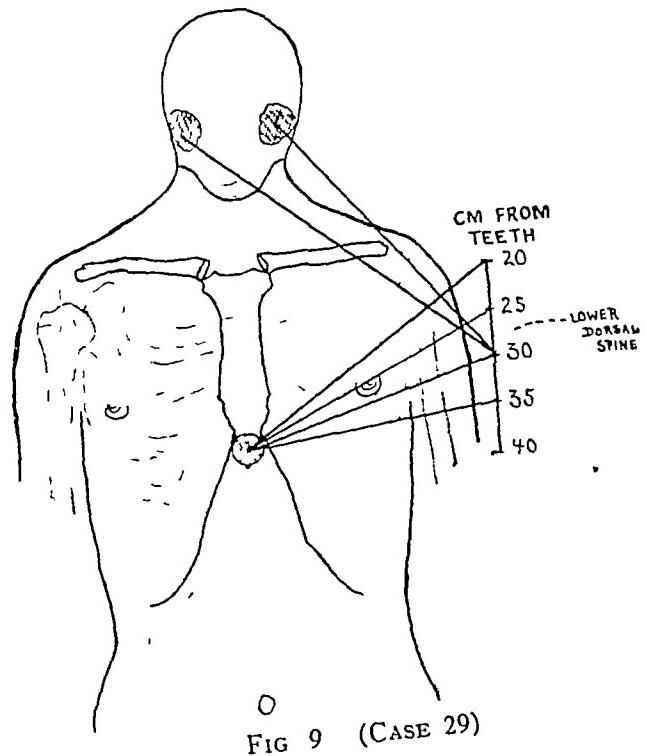


FIG 8 (CASE 34)

PAIN TSOPHAGUS

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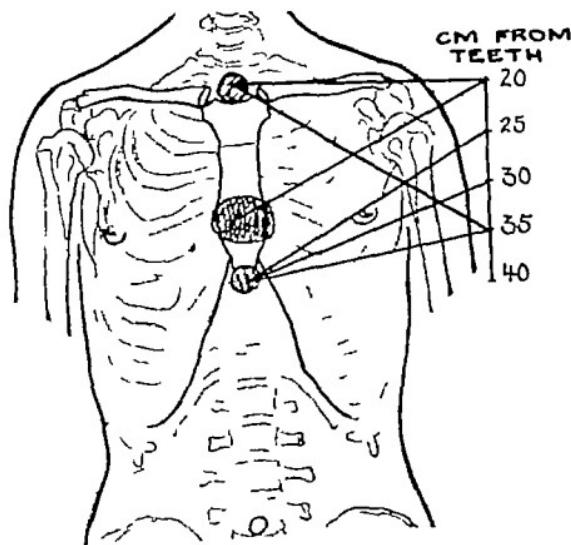


FIG 11 (CASE 14—SECOND TEST)

## THE INTENSITY OF THE STIMULUS NECESSARY TO PRODUCE PAIN

One object of the present work was to use standard stimuli so that differences in sensitiveness in different people could be determined. To this end air was introduced into the standard balloon at a constant rate of 2 cc per second. The results were however so variable that no really quantitative information was obtained. But in most of the subjects pain was felt after 15 to 25 cc of air had been introduced with a resulting pressure of from 80 to 150 mm Hg. Two typical cases are shown in Table III. An attempt was made to correlate the

TABLE III  
*Amount of air and pressure necessary to produce pain*

Level of balloon from teeth	Case 6		Case 8	
	Pain felt with air		Pain felt with air	
	Volume	Pressure	Volume	Pressure
cm	cc	mm Hg	cc	mm Hg
20	18	140	20	100
25	19	130	14	90
30	26	140	15	90
35	25	140	22	90

12	Pain and fullness in epigastrum after eating (cancer of stomach)	Unlike spontaneous
16	Indigestion and "heart-burn"	<i>Exactly like spontaneous</i>
22	Pain in upper abdomen $\frac{1}{2}$ to 1 hour p c Relieved by vomiting (gallstones)	Unlike spontaneous
26	Epigastric distress (duodenal ulcer)	Unlike spontaneous
27	Epigastric distress (duodenal ulcer)	<i>Pain produced at xyphoid is identical with the spontaneous distress</i>
28	Indigestion—discomfort radiating to left shoulder	<i>Exactly like spontaneous pain</i>
31	Epigastric distress (duodenal ulcer)	Unlike spontaneous pain
35	Epigastric distress (duodenal ulcer)	<i>Like spontaneous pain but milder</i>

In five of these thirteen cases inflation of the esophagus reproduced the patient's spontaneous discomfort. It seems highly likely, therefore, that some of the epigastric discomforts encountered clinically and the symptoms classed as "heart-burn" may originate in reflex contractions or spasms of the esophagus, a view which has already been proposed by others.

#### NATURE OF THE REFERRED PAIN

The sensations which have been described in the preceding sections might conceivably originate locally at the site of the stimulus or they might be referred to the superficies by way of vagus or sympathetic. We soon abandoned the idea of local pain in view of the lack of correlation between the site of the balloon and the position of the pain and because of the variable results from repeated stimuli at a given point. The surface area which includes all pain referred from the esophagus is innervated from the 3rd cervical to the 7th or 8th dorsal segments. It seems highly likely that the sensations produced over sternum, anterior chest wall and back are referred via the sympathetic to corresponding skin segments. The explanation of the suprasternal notch pain which occurred so commonly is less clear. If of sympathetic origin the 3rd cervical segment would be implicated and if so it seems remarkable that pain in the arms (C4 to D3) never occurred. The writers do not feel that their understanding of the physiology of the autonomic system is sound enough for them to pursue the discussion further, let it be sufficient to record the facts.

The possibility of distension of the esophagus at any one point

setting up reflex spasm elsewhere along the tube had to be considered as a possible explanation of pain which appeared at sites remote from the stimulus. We have no exact data as to this mechanism but it seems an unlikely one inasmuch as pain wherever produced invariably disappeared instantaneously as soon as the balloon was deflated.

#### DISCUSSION

The experiments described above show that it is possible to produce referred pain by inflation of small balloons in the esophagus. Such pain occurred, contrary to the findings of Hertz and of Payne and Poulton, without relation to the position of the bag in the esophagus and over a wide area of the torso and occasionally in the neck or face although the suprasternal notch and the xyphoid region were sites of predilection. The experimental findings were especially illuminating in connection with what has been observed in patients with esophageal disease. A recent case of carcinoma of the esophagus for example with the stricture just above the cardia complained after taking food of distress confined to the suprasternal notch and relieved by vomiting. In other clinical cases pain has been confined to the back as in some of the experimental subjects. Of especial interest were the patients with spontaneous epigastric distress associated with peptic ulcer whose symptoms were reproduced by inflation of the esophagus (Table V), a finding which suggests that reflex spasm of the esophagus may play a part in the production of "ulcer indigestion" in certain cases. At any rate familiarity with the areas over which pain may be referred experimentally from the esophagus is clearly useful in interpreting the diagnostic problems of clinical cases. With regard to the type of the referred sensations the vagueness of the descriptions is noteworthy and agrees with our experience in eliciting histories from patients. One evidently deals with an elementary form of discomfort which can not be accurately defined. It is of interest that sensations akin to clinical "heart burn" were produced in a number of instances obviously regurgitation of acid played no part here and this too agrees with clinical experience that heart burn may occur in patients with absence of acid in the gastric secretions. The "burning" may therefore be a characteristic of the referred sensation quite apart from the back flow of acid into the esophagus which of course occurs in many patients.

From the standpoint of exact physiological observation the results of the present study are disappointing, indeed we question whether phenomena of the sort with which we were dealing lend themselves to quantitative exploration. With every effort to standardize stimuli and to make the experimental conditions as objective as possible great variation occurred in the effects induced in the same subject on repeated tests—variations which occurred almost from moment to moment and which depended not only on psychic factors, but on the ever changing conditions of tonus and motility which are encountered in a hollow viscus.

#### SUMMARY

1 Inflation of small balloons in the esophagus produces referred sensations (pain)

2 The site of the referred pain bears no relation to the position of the balloon but occurs especially at the xiphoid or in the suprasternal notch, and less often over the anterior chest wall or in the back. Rarely it was felt in the neck or face and in two cases nausea was produced.

3 The character of the sensation was crude and often could not be accurately described or delineated by the subject. It frequently was identical with spontaneous "digestive" discomforts.

4 No relation was made out between the subject's general susceptibility to pain and the ease with which referred pain was produced.

5 The bearings of these observations on clinical diagnosis are discussed.

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## EXPERIMENTAL REFERRED PAIN FROM THE GASTRO-INTESTINAL TRACT PART II STOMACH DUODENUM AND COLON

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### INTRODUCTION

In the preceding paper of this series (3) observations on the pain resulting from inflation of the esophagus by small balloons were described. The general methods and purposes of the investigation were also outlined. The present communication deals with similar studies of referred sensation from the stomach, duodenum and colon.

### STOMACH

*Methods and material* A large balloon of thin rubber, so shaped that on inflation it assumed the general shape of the stomach, was constructed about the distal part of a mercury weighted stomach tube. The tube was passed into the stomach and then inflated by means of a large luer syringe fitted with a valve so that the amount of air introduced could be measured. The stomach tube was also connected with a U mercury manometer to measure pressure (see Fig. 1).

Sixteen subjects were studied. They were hospital patients and included people with and without stomach disorders. The following points were analyzed (1) the amount of inflation necessary to produce pain (2) the location of the pain, (3) the character of the pain (4) the relation of pain to spontaneous sensations and to that produced by inflation of esophagus and duodenum and (5) the mechanism of the induced sensations. The results are summarized in Table I.

*The character of the sensations* The main features of the referred sensation from gastric inflation was its indefinite quality. It was almost impossible to get exact descriptions from the subjects but on the

whole the sensations resembled those encountered ordinarily from loading the stomach—feelings of fullness, tightness or pressure more or less of a painful element superimposed. In several cases were reflex attempts to rid the stomach of the large foreign body means of belching, or violent nausea and retching supervened. I

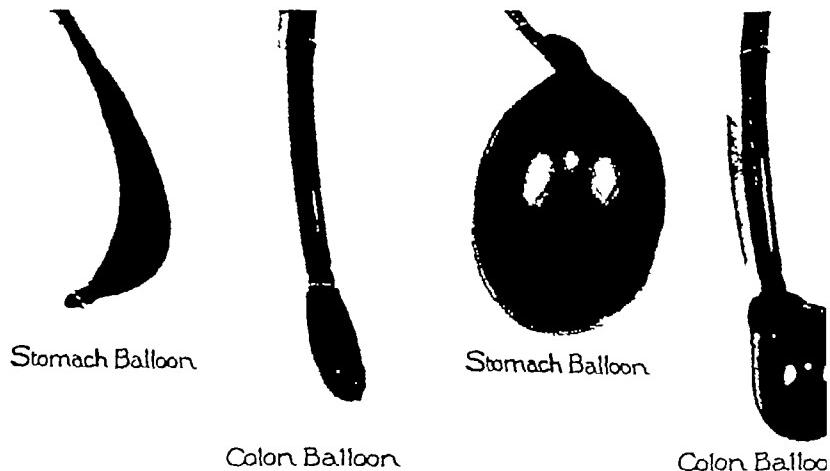


FIG 1 STOMACH AND COLON BALLOONS—INFLATED AND COLLAPSED

of interest to observe how violently sick the subject could be as a result of a purely mechanical stimulus. When more than 600–700 cc of air were introduced the stomach outline began to stand out in visible fullness, in no case was there visible peristalsis. Case 14 is of interest insofar as no definite sensation resulted from distension with 1500 cc of air. In other cases discomfort resulted (see Table I) with 200 to 400 or 500 cc. Except in Case 14, who seemed to be generally hyposusceptible to pain, no relation was found between the threshold stimulus and the patient's general nervous make-up or the presence or absence of disease of the stomach. Cases 12 and 15, for example, with peptic ulcer responded very slightly to inflation.

When inflation was started the pressure in the bag rose rapidly to about 40 mm Hg and then usually remained at about this level. Apparently the stomach dilated readily on further introduction of air without exerting much increased pressure.

*The location of the induced sensations.* Inflation of the stomach gave sensations which were much less sharply localized than those from

TABLE I  
*Abstract of case data and results of inflation of stomach*

Case number	Minimum amount of air necessary to produce sensation	Site of sensation	Character of sensation	Results of further inflation	Remarks
1	1400 cc.	Just above and to right of umbilicus	Indefinite sensation of fullness. No pain		Chronic gastritis and anacidity. Patient's spontaneous complaint is that his stomach feels full as soon as he begins to eat. This is of interest in view of the large amount of air necessary to produce referred sensation
2	1500 cc.	Epigastrium	General feeling of fullness. No pain		
3	400 cc.	4 cm below xyphoid and lower	Sickish gas pain	600 cc.—burning sickish full feeling like when he over eats. 700 cc.—nausea 1000 cc.—belching 1200 cc.—diffuse colic between xyphoid and umbilicus	Clinical diagnosis. Hyperacidity and indigestion. Low threshold for referred pain. Pain from stomach entirely unlike that from esophagus (see <i>esophagus series—Case 16</i> )
4	700 cc.	Half way between xyphoid and umbilicus radiating up towards left costal margin	Painful tight feeling	1000 cc.—hurts in epigastrium 1100 cc.—severe pain—same place	A woman of 30 years who complained of indefinite abdominal pain. Does not describe artificial pain well but thinks it resembles her spontaneous pain and the pain from inflation of esophagus

TABLE I (continued)

Case number	Minimum amount of air necessary to produce sensation	Site of sensation	Character of sensation	Results of further inflation	Remarks
5	400 cc	Epigastrium—half way down to umbilicus and up under costal margin	Diffuse discomfort—"Pressure"	700 cc—pressure worse in same location followed by belching and violent nausea and retching	A woman of 34 years with gallstones and epigastric pain $\frac{1}{2}$ to 1 hour p.c relieved by vomiting Artificial pain in same place and much like spontaneous pain
6	400 cc	Above and to left of umbilicus	"Bloating"—no pain	700 cc—slight pain below xiphoid 1100 cc—"pressure" half way between xiphoid and umbilicus 1400 cc—painful fullness in epigastrium 1500 cc—same	A man of 41 years with subacute combined sclerosis and anacidity No digestive symptoms
7	500 cc	Half way between xiphoid and umbilicus	" Burning, gnawing pain"	Sensation worse as pressure increased—at 1400 cc—nausea	A man of 31 with duodenal ulcer Says artificial pain resembles his spontaneous symptoms Also similar to esophageal pain
8	200 cc	Half way between xiphoid and umbilicus	"Slight pressure"	400 cc—marked "pressure" 700 cc—severe cramp like pain in same spot—felt like "cramps when bowels are going to move"	A woman of 26 years with cardiospasm Artificial pain unlike spontaneous discomforts

TABLE I (continued)

Case number	Minimum amount of air necessary to produce sensation	Site of sensation	Character of sensation	Results of further inflation	Remarks
9	400 cc.	Left anterior axillary line at level of 7th rib	A kind of ache	On each increase of pressure had severe <i>pains in head</i> —at 1000 cc. severe pain along left costal margin	A man of 54 years with an obscure pain below left costal margin. Artificial pain in same place but unlike spontaneous
10	500 cc.	Half way between umbilicus and xyphoid	A painful fullness as when she has eaten food and it disagreed Not cramplike	600 cc—pain very severe	A woman of 56 without digestive complaints. Stomach pain unlike esophagus pain
11	400 cc.	Half way between umbilicus and xyphoid	Feeling of gas	1100 cc—diffuse pain below left costal margin	A woman of 32 years with indigestion Spontaneous symptoms unlike either induced stomach or esophagus pain
12	400 cc.	Umbilical region	Pressure	1000 cc—pain about 10 cm to left of umbilicus like a side ache	A man of 70 years with duodenal ulcer Says he often has side ache like the induced pain It is unlike esophageal pain
13	500 cc.	Epigastrum	Fullness—wants to belch	900 cc.—full can 'hardly get breath' No pain 1300 cc—painful feeling all over region from xyphoid to umbilicus	A man of 66 years with catarrhal jaundice. Unlike esophageal pain

TABLE I (*continued*)

Case number	Minimum amount of air necessary to produce sensation	Site of sensation	Character of sensation	Results of further inflation	Remarks
14	700 cc	Half way between xiphoid and umbilicus	Very slight "pressure" No pain	1500 cc—no sensation except vague general abdominal fullness	A man of 34 years remarkably insensitive to pain Practically no reaction to marked inflation See Case 34—esophagus series
15	1100 cc	Just below xiphoid	Felt "stuffed"	1500 cc—same, a little more marked	A man of 41 years with duodenal ulcer Induced sensation not like spontaneous discomfort or like esophageal pain
16	500 cc	Epigastrum	"Feels full all over"	1200 cc—fullness and discomfort in epigastrum	A man of 54 years with peptic ulcer of esophagus Induced sensation unlike spontaneous or esophageal pain

esophagus or duodenum. The subject usually placed his whole hand over the general area which was affected, but the sensation was usually described as deep and not on the surface. The dots in Fig 2 show the centers of the areas indicated in the various cases, the total distribution of sensation corresponded roughly with the rectangular area. In no

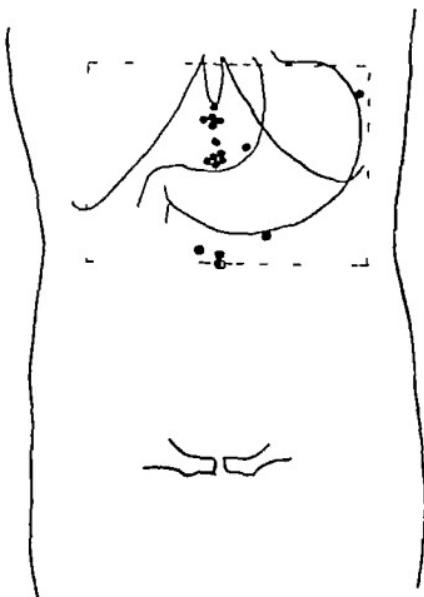


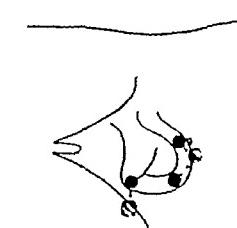
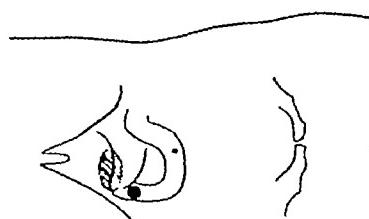
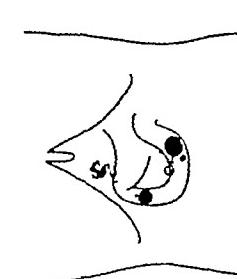
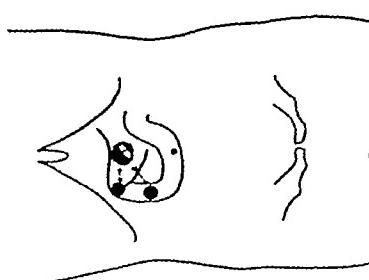
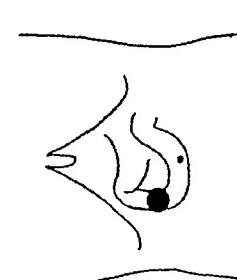
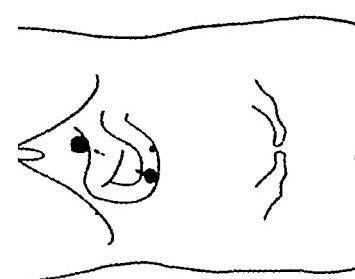
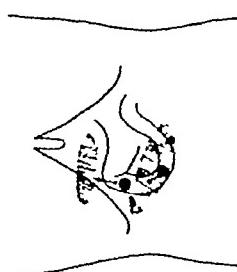
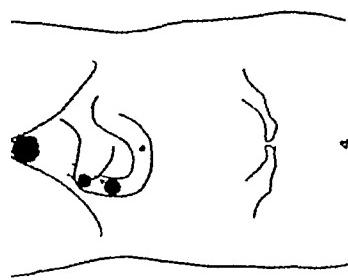
FIG 2 EACH DOT INDICATES THE CENTER OF THE AREA OF REFERRED SENSATION FROM INFLATION OF THE STOMACH IN SIXTEEN CASES

case did inflation of the stomach give pain much below the umbilicus or above the xiphoid with the exception of Case 9 who on each step in the inflation had severe pain in the temporal regions which was immediately relieved by releasing the pressure.

*Relation of induced sensations to spontaneous discomforts and to esophageal and duodenal pain.* In several cases (Cases 4, 5, 7, 12) the induced sensations were said by the patient definitely to resemble spontaneous discomforts. In Cases 4 and 7 the induced stomach sensation resembled that produced by inflation of esophagus. In other instances (see below) there was a resemblance between esophageal and duodenal pain. The point of interest and of clinical importance is the difficulty of identifying the site of the stimulus from the location of the referred sensation.

3

## PAIN STOMACH, DUODENUM AND COLON



200 CALORIES  
240 CALORIES  
250 CALORIES  
260 CALORIES

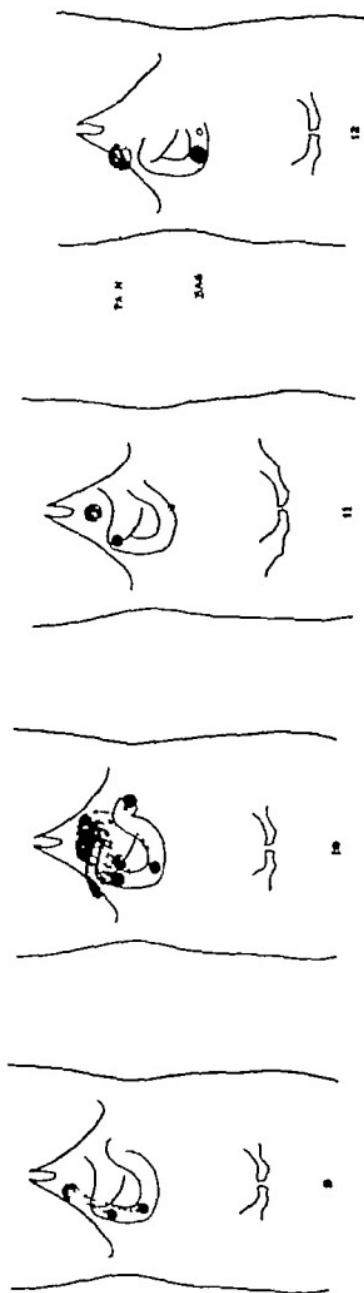


FIG. 3 REFERRED PAIN FROM INFLATION OF DUODENUM IN TWELVE CASES. The position of the bag is indicated by the solid black dots; the resulting sites of referred pain by the shaded areas.

*Mechanism of the referred sensations* The abdominal surface area implicated by the referred sensations corresponded to the 7th, 8th and 9th dorsal segments We interpreted the sensations as referred via the sympathetic However, nausea and retching, and in Case 9 the pains in the head, seemed clearly to be reflexes through the vagus

#### DUODENUM

*Literature* Ivy, Vloedman and Keane (1) inflated the duodenum in three normal men with 50 cc of air and produced nausea and chilliness Strouse and Shamberg (2) carried out similar experiments in a larger series of people In patients without abdominal disease they concluded that pain was localized at the position of the balloon whereas in cases of abdominal disease the results were variable or the patient's spontaneous pain might be reproduced As will be seen below our experiments yielded somewhat different results

*Methods and material* A duodenal tube fitted with a balloon at its tip was used The tube was passed in the usual manner and its position was verified under the fluoroscope In a good many instances the balloon slipped back into the stomach as soon as inflation was begun so that only about one third of the experiments were successful However, in twelve cases the procedure was carried out satisfactorily under fluoroscopic control The exact position of the balloon, the amount of air necessary to produce sensation and the pressure in mm of mercury, the character of the pain and other symptoms and the location of induced sensations were recorded The subjects included hospital patients with and without digestive symptoms

*Results* The main features of the observations are summarized in Table II and in Fig 3

*The character of the referred sensations* The character of the referred sensations was similar to that obtained on inflation of the esophagus and stomach—deep seated more or less indescribable unpleasant feelings into which entered, in various cases, elements of pressure, fullness, burning, aching or colic In a general way the induced discomforts resembled those complained of by patients with indigestion, peptic ulcer, gallbladder disease or other abdominal disorders They presented no specific features In several cases nausea and vomiting were induced On relieving the inflation the referred sensation always disappeared instantaneously and there were no after effects

TABLE II  
Summary of data from inflation of duodenum

Case number	Position of bag in duodenum	Volume of air and pressure necessary to produce sensation		Character of sensation	Location of sensation	Remarks
		Volume	Pressure			
1	2nd portion	150	100	A severe sharp pain which doubles you up	See Fig. 3	A man of 60 years with an acidity Never any similar spontaneous pain
2	3rd portion pyloric canal	80 40	120 100	A severe ache' same	'	A man of 66 years with catarrhal jaundice. Never any similar spontaneous pain
3	3rd portion	75		A full cramplike feeling'	'	A man of 41 years with duodenal ulcer Induced pain resembles spontaneous pain in character and location
4	1st portion pyloric canal	50 50	70 70	Pain same	'	A man of 54 with peptic ulcer of esophagus and attacks of epigastric pain In induced pain resembles both his spontaneous discomfort and induced esophageal pain
5	3rd portion	75	70	A hot sticking pain	See Fig. 3	A man of 60 years without spontaneous abdominal symptoms. Induced pain did not resemble esophageal pain Out standing reaction from both duodenum and esophagus is nausea and vomiting
	2nd portion	100	90	Retching and vomiting		
	pylorus	50	100	Severe pain same place		
6	3rd portion	50	85	Severe pain nausea and vomiting	'	
	1st portion	75	85	Pain — apparently a sharp colic		

TABLE II (*continued*)

Case number	Position of bag in duodenum	Volume of air and pressure necessary to produce sensation	Character of sensation	Location of sensation	Remarks
		Volume mm Hg			
7	2nd portion	" 200	No sensation in spite of great inflation of bag which reached a diameter of 7 cm	See Fig 3	A man of 36 years with tape worm No spontaneous abdominal symptoms
8	3rd portion	50	"A burning cramp"—nausea same	" " "	A man of 27 years with "indigestion" Induced pain unlike spontaneous symptoms
	1st portion	50	60		
9	2nd portion	40	"Dull ache"	" " "	A man of 21 years with tape worm No spontaneous digestive symptoms
		80	Severe sharp pain which doubled him up		
	1st portion	80	60		
10	4th portion	120	Dull pain "like pressing on a sore"	" " "	A man of 52 years with old duodenal ulcer and catarrhal jaundice No indigestion recently Induced pain similar to old ulcer pain and in same region
	3rd portion	140	Same type of pain		
	2nd portion	130	A pressure feeling "like something stretched too big"		
		80			
11	pyloric canal	200	"Dull pressure"	" " "	A man of 65 with duodenal ulcer Same location as "ulcer pain" but not so severe Thinks it is same kind of pain
12	3rd portion	125	Severe pain	" " "	A man of 34 convalescent from pneumonia No spontaneous digestive symptoms

*The location of the referred sensations* Fig 3 shows the location of the referred sensations in relation to the position of the balloon in each case and Fig 4 shows the center of pain in all the cases and the

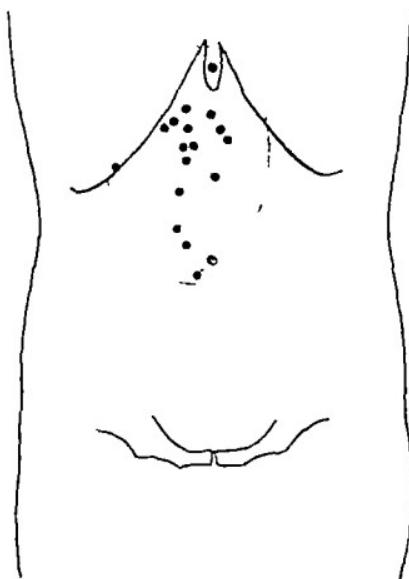


FIG 4 DOTTED CIRCLE INDICATES TOTAL AREA IMPLICATED BY REFERRED PAIN FROM THE DUODENUM Each dot indicates the site of pain from a single inflation

total area which was implicated. It is seen that inflation of the duodenum may give pain from the xyphoid to the umbilicus although the right upper quadrant was most frequently referred to in these tests. We were unable to confirm Strouse and Shamberg's observation that in normals the pain was referred to the site of the balloon in contrast to other forms of reference in patients with intraabdominal disease; indeed in our cases there was no correlation of any sort. For the most part the referred pain was sharply localized (see diagrams) in contrast to the widely diffused sensations from the stomach. However the stomach and duodenal areas overlapped (see Fig 7).

*The degree of inflation necessary to produce pain* Table II shows that pain appeared in different cases with variable degrees of inflation—from 40 cc to 200 cc of air. The pressure in the bag also varied con-

siderably Case 7 had no sensation even after the introduction of 200 cc of air It was impossible to correlate these variations with any clinical features such as presence or absence of disease of the duodenum, certainly the three cases of duodenal ulcer were not specially sensitive to inflation

*The relation of induced to spontaneous pain and of gastric esophageal and duodenal pain* Table III shows in summary these relations In several cases inflation of duodenum reproduced the patient's spontaneous symptoms, in some instances these also seemed identical with induced pain from the esophagus Obviously one should not go too far in drawing conclusions from statements of patients as to subjective sensations, but a point of great clinical importance is brought out insofar as one clearly must be very cautious in diagnosing the site of origin of a pain from its situation alone

#### COLON

Great difficulties were encountered in introducing the balloon (see Fig 1) into the large bowel and our observations are confined to inflations of the left colon and sigmoid In order to prevent coiling of the tube in the rectum, which otherwise invariably occurred it was necessary to introduce the tube into the sigmoid through a proctoscope and then to attempt to pass it further Successful observations, with the position of the bag checked by fluoroscopy, were obtained in nine patients with miscellaneous disorders, and except for Case 8 without bowel disease We had found no reference in the literature to similar experiments on the intact bowel Since this paper was completed we have learned that Chester M Jones, of Boston, has presented a communication to the Cosmopolitan Club at their meeting on February 27, 1930, dealing with the problem of referred pain from the bowel as the result of inflation by means of balloons From personal communication with Dr Jones it appears that he has obtained results essentially similar to ours It should also be pointed out that his present studies are the outgrowth of similar work on the esophagus which he reported several years ago at the meeting of the American Society for Clinical Investigation (5) Our work is therefore to be regarded as consequent and not antecedent to his

*Results* The results are summarized in Table IV and Figs 5 and 6

TABLE III  
*Relation of induced to spontaneous pain*

Case number	Spontaneous discomfort	Induced pain		
		Esophagus	Stomach	Duodenum
1	None		All different	
2	None	Like duodenal	Unlike esophageal or duodenal	Like esophageal
3	Epigastric discomfort	Like duodenal and spontaneous discomfort	Unlike spontaneous or induced pains	Like esophageal and spontaneous discomfort
4	Epigastric discomfort	Like duodenal and spontaneous discomfort	Unlike spontaneous or induced pain	Like esophageal and spontaneous discomfort
5	None	Nausea and vomiting		Nausea and vomiting
8	Epigastric discomfort	Unlike duodenal or spontaneous pain		Unlike esophageal or spontaneous pain
10	Epigastric discomfort			Like spontaneous discomfort
11	Epigastric discomfort			Like spontaneous discomfort

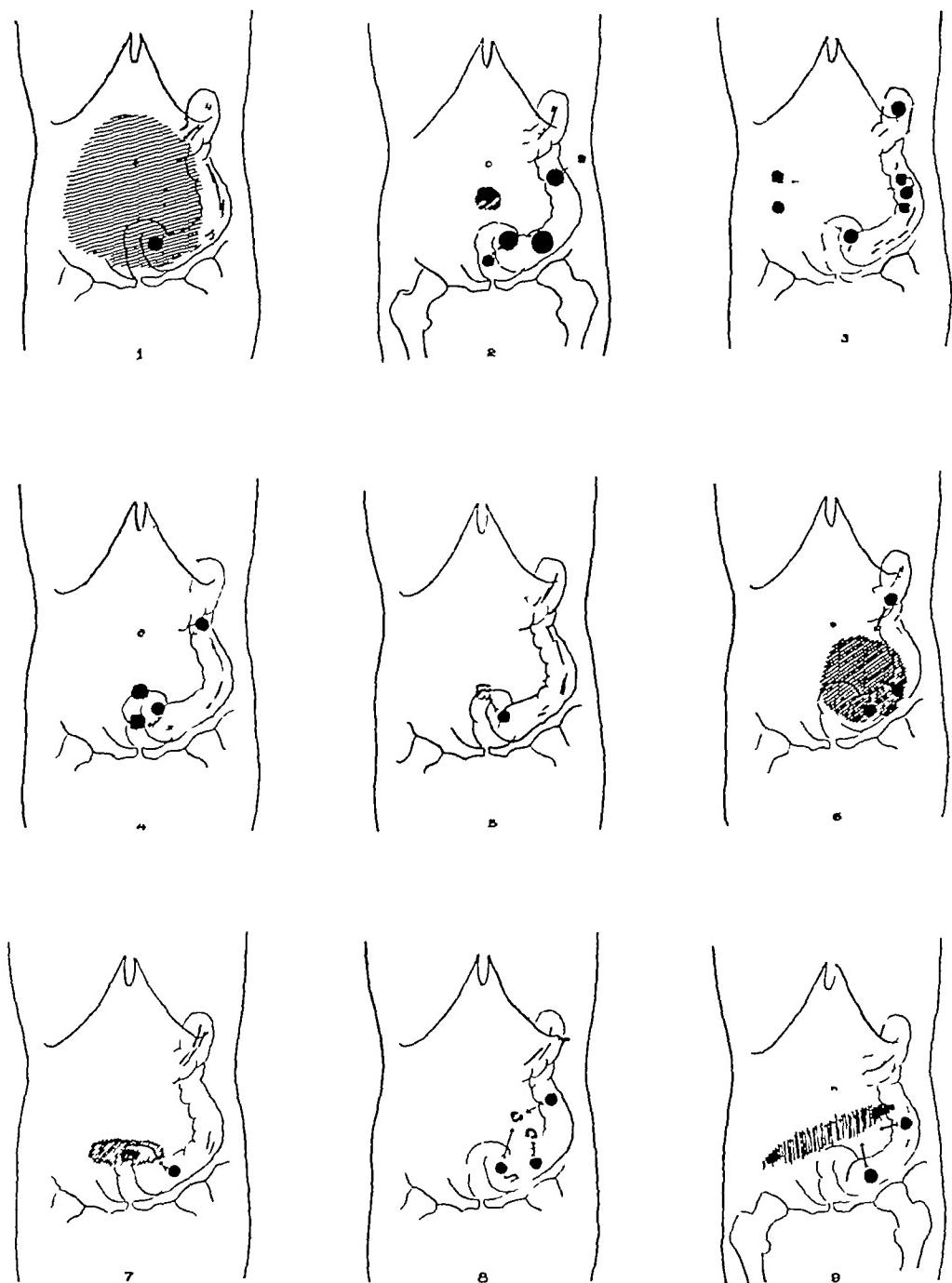


FIG 5 REFERRED PAIN FROM INFLATION OF COLON AND SIGMOID IN NINE CASES The solid black dots show the position of the bag, arrows point to shaded areas which indicate the resulting areas of referred pain

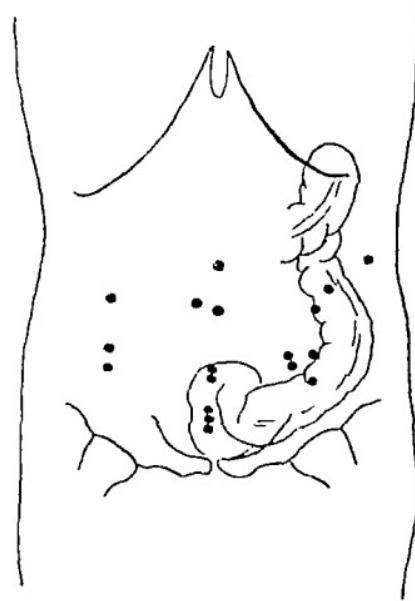


FIG 6 REFERRED PAIN FROM COLON. Each dot indicates the center of referred pain from a single inflation.

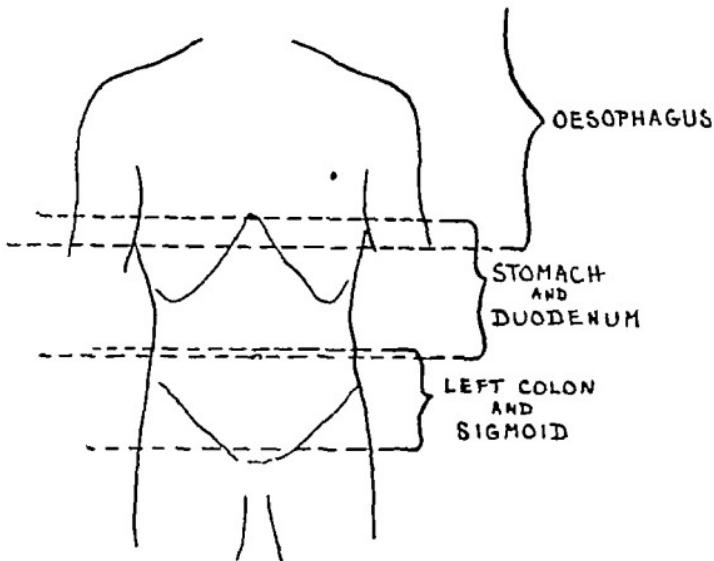


FIG 7 AREAS OF REFERRED PAIN DEMONSTRATED BY INFLATION OF BALLOONS IN THE GASTRO INTESTINAL TRACT

TABLE IV  
*Summary of data from inflation of colon*

Case number	Amount of air	Site of balloon	Character of pain	Site of pain	Remarks
1	" 500	Sigmoid	Diffuse, transient pain as air was introduced	See Fig 5	Angina pectoris
2	150	Half way up left colon	"Pressure pain"	" "	Aortic insufficiency
	150	Sigmoid (mid)	Same		
	150	Sigmoid (lower)	Same		
3	150	Splenic flexure	"Pain"	" "	Normal
	150	Half way up left colon	Same		
	150	Sigmoid	Same		
4	200	Splenic flexure	Pain like "before he has to go to stool"	" "	Indigestion—no bowel trouble
	150	Sigmoid	Same	" "	
5	?	Sigmoid	"Pain"	" "	Normal
6	100	Splenic flexure	Severe pain—"a kind of cramps"	" "	Chronic constipation
	100	Lower end of colon	Same		
	100	Sigmoid	Same		
7	20	Sigmoid	Pain "like an awful stomach ache"	" "	Peptic ulcer? No bowel trouble
8	100	Half way up left colon	"Like gas pain"	" "	Gastritis and anacidity
	100	Sigmoid			Recurring diarrhea with gas pains and indigestion
	300	Recto sigmoid			Induced pain resembled spontaneous discomfort
9	100	Lower colon	A "gassy distended feeling"	" "	Pleurisy—no bowel symptoms
	100	Sigmoid	Same		

Referred sensations of the same general sort as those originating in the stomach or duodenum were obtained—more or less indescribable deep discomforts involving elements of fullness, pressure or colic and obviously related to sensations normally experienced in connection with movement of the bowel. As a rule pain appeared with about 100 to 200 cc of air but in Case 7, 20 cc sufficed and in Case 1, 500 cc were necessary. We were unable to correlate these variations with any other features of the case. In some instances the pain was sharply localized as regards surface distribution as in Case 5, in others there was a wide indefinite area as in Cases 1 and 6. In Fig. 6 each dot indicates the center of the area of referred sensation from a single inflation. It is seen that the whole area from umbilicus to pubis is implicated and that there was no definite relation between the site of the stimulus and the site of referred pain. Of special interest for example, was Case 9 who with the bag in the descending colon felt pain in the right lower quadrant. In some cases the bag could be moved quite a distance without altering the position of the pain (Case 3). It is evident how uncertain it would be clinically to draw inferences as to the location of a lesion from the site of pain alone. As soon as the bag was drawn from the sigmoid into the upper rectum and inflated there a new type of sensation resulted, namely a pain low in the back similar to that experienced when the bowels are about to move.

#### SUMMARY AND DISCUSSION

These experiments show in brief that by inflating various parts of the gastro intestinal tract with balloons referred sensations may be produced which fall under the general heading of pain. More in detail, these sensations may be described as deep seated fundamental more or less indescribable forms of discomfort into which enter elements classed by the subject as pressure, fullness, distension, tightness, cramps or burning. In many cases the subject recognizes the induced sensation as similar to spontaneous discomforts encountered in his past experience and likens them to the sensations of overeating or indigestion, of desiring to go to stool, etc. For the most part the induced sensations seemed to be referred to segmental areas via the sympathetic, although in some cases nausea, vomiting and remote pain as in the head or face probably represented vagal reflexes.

The main object of our studies was to throw light on clinical problems particularly with reference to the relation of site of pain to its point of origin, and it was brought out almost consistently that the superficial distribution of the referred sensations was remote from the site of the stimulus. One may mention, for example, pain in the suprasternal notch from inflation of the lower end of the esophagus, pain in the gallbladder region from inflation of the 3rd portion of the duodenum and pain in the lower mid-abdomen from inflation of the splenic flexure. While the total areas of referred sensation from esophagus, duodenum and colon are different, they overlap to some extent (see Fig 7) so that at times it may be difficult to tell, for example, whether pain originates in the esophagus or in the upper abdominal viscera.

These facts seem to sustain the thesis laid down in the preceding paper (3) that symptoms of this sort occurring in patients do not by themselves enable one to make a diagnosis of the underlying disorder. This concept, even if not generally accepted at present, is not a new one and was championed by Sir James Mackenzie "Attempts are continually being made to classify affections of the stomach, and the lack of agreement in these classifications is merely due to the fact that attempts are made to differentiate what can not be differentiated. This will be realized when the nature of stomach symptoms is considered. Apart from some characteristic vomits (blood, mucus) and certain changes indicated in the position of the organ (and these refer only to a minute proportion of the cases), all the symptoms are of a reflex nature, pain, cutaneous and muscular hyperalgesia, muscular contraction, vomiting and air suction. As any adequate stimulus may suffice to produce these symptoms, and as this adequate stimulus may arise from the most various causes, trivial or severe, it follows that there is a great similarity in the symptoms in diseases of the most varied kinds" (4). If all this be true it would seem to be sounder practice to admit the limitations of "digestive symptoms" in diagnosis and to realize that unless more conclusive evidence can be obtained by x-ray study or in other ways it is unwise to set up criteria which actual experience shows to be unsound.

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## THE BLOOD VOLUME IN HYPERTHYROIDISM

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Thyrotoxicosis is almost invariably associated with a certain degree of cardiovascular disturbance. Tachycardia is always one of the first signs of this malady. Demonstrable enlargement of the heart frequently follows. Cardiac arrhythmia, especially auricular fibrillation, and congestive heart failure often constitute the important part of the clinical picture during the later part of the course of the disease. Occasionally the patient's death may be directly attributable to cardiac insufficiency. Although various views have been put forward, the cause and nature of these circulatory derangements remain incompletely understood. Attempts to find characteristic histological changes in the myocardium have been without success. The isolated reports of damage to the heart muscle (1) (2) (3) (4) give no convincing evidence that the lesion was specific. That the thyroid heart is not a permanent condition has been recently emphasized by Thomas (5). One of the patients reported by this author died from heart failure, but presented no significant myocardial damage on postmortem examination. On the other hand investigations into the physiological pathology of the disease have been less disappointing. Years ago Plesch (6) demonstrated an increased minute volume in the patients suffering from exophthalmic goiter. Stewart (7) by his special technic found in Graves' disease an exceptionally large blood flow in the hands and this he thought was in agreement with the flushing of the skin which is commonly seen. More recently the cardiac output in hyperthyroidism has aroused a renewed interest (8) (9) (10) (11) (12). In spite of the widely different methods employed, all investigators with the single exception of Rabinowitch and Bazin (9) have come to the same conclusion as that reached by Plesch. Blumgart, Gargill and Gilligan (13) studied 13 thyrotoxic patients and showed that the

speed of blood flow through the lungs was much faster than the average normal speed of flow. The velocity of the venous blood flow from the arm to the heart was likewise greatly enhanced. It would thus seem that the cardiac disturbance in thyroid disease may merely represent the effect of prolonged overloading and fatigue of the heart muscle. Inasmuch as the total circulating mass plays an important rôle in hemodynamics an inquiry into this factor should assist greatly in understanding the magnitude of the extra load imposed upon the heart. Blotner, Fitz, and Murphy (14) have already studied the total blood count in this disease. The present communication deals with the blood volume findings in hyperthyroidism and their subsequent changes after partial thyroidectomy.

#### EXPERIMENTAL

The blood volume was determined by the carbon monoxide method according to the technic reported previously (15). This is a simple and reliable method with a maximal error of less than 5 per cent. It has the special merit of giving constant and consistent results in the same individual with repeated measurements, thus obviating the objection of an "immunity" effect inherent in the use of dye as pointed out by Lindhard (16). Four normal subjects were observed repeatedly under identical conditions as regards position, environmental temperature, physical activity, and the state of alimentation. Their blood volumes determined by the carbon monoxide method remained practically the same on these different occasions (Table 1).

The routine procedure in the study of the patients was as follows. In measuring the blood volume basal condition was not maintained, but the technic was carefully standardized so that all determinations were done in the afternoon about three hours after lunch, with the patient in recumbent posture, and after thirty minutes of complete rest. The basal metabolic rate was obtained by the Tissot method. The hematocrit readings, red blood cell count and estimation of the oxygen capacity were carried out by the usual methods. In selecting the patients for investigation only frank cases of hyperthyroidism were included. Border line cases and patients with proved organic heart disease were not included.

In all, 21 subjects were studied. The basal metabolic rate ranged

TABLE 1  
*Blood volumes of 4 normal subjects on repeated measurements (CO method)*

Subject	Date	Weight	Surface area	Blood volume		
				Total	Per kilogram	Per square meter
H C. C.	September 8 1928	kgm 64.0	square meter 1.75	cc. 4230	cc. 66.1	cc 2418
	November 5 1928	64.6	1.76	4170	64.5	2370
	December 5 1928	64.6	1.76	4185	64.7	2370
	February 10 1929	65.0	1.77	4220	65.0	2384
C T H	April 24 1928	65.8	1.79	4590	69.8	2565
	June 31, 1928	64.9	1.78	4550	70.1	2557
S H L	March 13, 1928	69.7	1.87	4670	67.0	2500
	July 26, 1928	68.0	1.86	4690	69.0	2520
S M L.	April 13 1928	52.6	1.59	3690	70.2	2320
	August 22 1928	52.0	1.58	3640	70.0	2300

between plus 23 and plus 109 per cent. All the subjects had clinical signs of exophthalmic goiter except one patient with a toxic adenoma. The data are summarized in Table 2. The average blood volume was 77.8 cc per kilogram of body weight and 2624 cc per square meter of body surface, both being definitely higher than the respective normal values<sup>1</sup>. In more than half of the patients the blood volume was higher than the maximal limit obtained in the normal series reported elsewhere (15). However, no definite correlation can be shown to exist between the extent of the increase in blood volume and the height of the basal metabolism. This is not surprising in view of the individual variation of both of these physiological factors in the normal subjects. The red blood cell count and the oxygen capacity seemed to fall within normal limits, except in Case 12 in which there was a definite reduction in hemoglobin. This is consistent with the belief that anemia is probably a rare event in uncomplicated hyperthyroidism (17). In no case was any appreciable polycythemia encountered.

<sup>1</sup> The blood volume of 16 normal adults determined by the same method averaged 66.6 cc per kilogram of body weight and 2474 cc per square meter of body surface (15).

## BLOOD VOLUME IN HYPERTHYROIDISM

TABLE 2

*Blood volume, oxygen capacity, hematocrit reading, red blood cell count and basal metabolism in 21 cases of hyperthyroidism*

Case number	Sex	Basal metabolic rate <i>per cent</i>	Blood volume			Red blood cells	Oxygen capacity	Cell volume
			Total	Per kilogram	Per square meter			
1	F	+55	3730	81.0	2590	4.22	17.3	37.5
2	F	+31	3315	73.0	2240	4.47	15.6	35.0
3	F	+51	3610	58.2	2099	4.93	20.9	43.5
4	F	+34	5120	76.4	2910	4.71	20.0	44.0
5	F	+73	4041	74.1	2574	5.00	19.1	43.0
6	F	+33	4780	74.0	2860		18.2	
7	F	+30	4880	69.0	2638	5.13	19.8	44.0
8	F	+47	3100	62.4	2095	4.51	14.7	
9	F	+54	4552	86.3	2958	5.28	20.1	
10	F	+68	3325	62.6	2190	5.28	18.5	43.2
11	F	+43	3250	69.2	2258	4.38	14.7	34.8
12	F	+74	4500	95.0	3100	4.44	12.8	34.8
13	M	+50	5100	81.0	2930		18.4	43.7
14	F	+40	3285	78.0	2570	4.68	16.2	
15	F	+23	3610	60.6	2175	4.33	17.5	
16	F	+48	3930	84.4	2730	4.31	16.5	37.8
17	M	+70	4170	91.2	2725	4.87	16.3	47.4
18	M	+61	5610	115.2	3635	5.20	18.5	
19	F	+109	3965	82.6	2680	5.09	16.5	40.0
20	F	+80	3120	72.4	2200	5.01	17.1	39.5
21	F	+34	5390	88.0	2950	5.14	20.6	47.0
Average		+53	4113	77.8	2624	4.79	17.6	40.9

These high values for the volume of blood in hyperthyroidism might have been argued to be only apparent since these patients had probably all lost weight and the circulatory volume relative to the body weight and the body surface would naturally be high. To settle this point it remained necessary to study these cases both during the course of the illness and after a cure was effected through partial removal of the diseased thyroid gland. This was possible in 15 patients in whom in addition to the initial determination subsequent examinations were carried out after the institution of treatment. The routine therapeutic procedure consisted of digitalization and administration of Lugol's

solution, followed, at an opportune time, by subtotal thyroidectomy. After the operation the patients were symptomatically relieved and their basal metabolic rates practically all returned to the normal level. The time between the operation and the subsequent determination of blood volume varied from 10 days to 5 months, but in the majority of cases the interval was fairly short so that little difference, if any, existed between the patient's weight before and after the surgical procedure. The results are given in Table 3.<sup>2</sup> In Table 4 the percentage decrease of the metabolic rate after the operation is compared with the corresponding figure for the blood volume. It may be seen that these two run quite parallel with each other. Only in Case 14 does this parallelism not seem to exist and it is interesting to note that this is the only patient in the series who had a toxic adenoma. This patient did not appear ill there was no tachycardia or cardiac enlargement and the only indication of disease was the elevated metabolism. Subtotal thyroidectomy was not performed for this patient. After the enucleation of the adenoma the metabolism fell but not the blood volume. In nearly all the cases while the diminution of the blood volume following the operation was definite and far exceeded the maximal error of the experimental method, it was less striking than the decrease in metabolism. This is probably because metabolism permits a greater deviation from normal and because the change in blood volume is not the only circulatory adjustment in hyperthyroidism. As a rule the total blood volume and the volume relative to the body surface showed a percentage fall of approximately the same magnitude.

That the observed postoperative change in the blood did not represent the immediate effect of the surgical interference was clearly shown in Case 9. This patient was followed for nearly two years after her operation. Her blood volume throughout this period remained practically the same and was always considerably lower than before the subtotal thyroidectomy.

Iodine in the form of Lugol's solution efficiently lowered the basal metabolism in all the cases. Of the five patients studied after the administration of this drug four showed a decisive drop in blood volume

<sup>2</sup> The first seven cases were studied through the kindness of Dr G. A. Harrop Jr. in the Johns Hopkins Medical Clinic to whom the author wishes to express his appreciation.

TABLE 3  
*Blood volume, basal metabolic rate, red blood cell count, oxygen capacity and cell volume in 15 cases of hyperthyroidism before and after treatment*

Case number	Date	Weight	Sur face area, sq m	Basal metabolic rate per cent	Blood volume			Red blood cells million	Oxygen capacity per cent	Cell volume per cent	Remarks
					Total	Per kilo- gram	Per square meter				
1	April 4, 1927	46.0	1.44	+55	3730	81.0	2590	4.22	17.3	37.5	Before subtotal thyroidectomy
	May 31, 1927	55.4	1.55	-10	3295	59.5	2125	4.35	16.8	39.5	After subtotal thyroidectomy
2	April 5, 1927	45.4	1.48	+31	3315	73.0	2240	4.47	15.6	35.0	Before subtotal thyroidectomy
	May 11, 1927	46.5	1.49	-15	2875	61.8	1930	4.97	15.2	37.0	After subtotal thyroidectomy
3	April 8, 1927	62.0	1.72	+51	3610	58.2	2099	4.93	20.9	43.5	Before subtotal thyroidectomy
	May 12, 1927	64.1	1.75	+2	3190	50.0	1823	4.19	15.8	40.5	After subtotal thyroidectomy
4	April 6, 1927	67.0	1.76	+34	5120	76.4	2910	4.71	20.0	44.0	Before subtotal thyroidectomy
	June 3, 1927	68.1	1.77	-6	4320	63.4	2440	4.69	19.4	44.0	After subtotal thyroidectomy
5	April 25, 1927	54.5	1.57	+73	4041	74.1	2574	5.00	19.1	43.0	Before subtotal thyroidectomy
	June 1, 1927	56.0	1.58	+7	3590	64.1	2272	4.84	18.7	41.5	After subtotal thyroidectomy
6	December 7, 1926	64.6	1.67		4780	74.0	2860		18.2		Heart failure
	December 17, 1926	59.3	1.60	+33	3970	66.9	2480	5.40	19.3	45.0	Before subtotal thyroidectomy
7	January 17, 1927	55.3	1.56	-14	3595	65.0	2300	5.01	16.0	37.0	After subtotal thyroidectomy
	February 3, 1927	70.7	1.85	+30	4880	69.0	2638	5.13	19.8	44.0	Before subtotal thyroidectomy
8	February 25, 1927	71.2	1.86	-16	4120	57.9	2215	5.17	19.4	40.0	After subtotal thyroidectomy
	June 4, 1927	84.3	1.98		4475	53.1	2260	4.73	20.1	47.0	Signs of hypothyroidism
9	October 31, 1928	49.7	1.48	+47	3100	62.4	2095	4.51	14.7	Before subtotal thyroidectomy	
	January 4, 1929	50.6	1.58	-15	2875	56.8	1843	4.50	17.8	After subtotal thyroidectomy	

TABLE 3 (continued)

Case number	Date	Weight	Sur face area	Basal metabolic rate	Blood volume			Oxygen capacity	Cell volume	Per cent	Remarks
					Total	Per kilogram	Per square meter				
9	March 1 1928	52.8	1.54	+5.5	4552	86.3	2953	5.28	20.1	On admission	
	March 13 1928	53.0	1.55	+1.2	3740	70.5	2410	5.44	17.4	After a course of Lugol's solution	
	March 31 1928	52.0	1.54	-2.0	3170	61.0	2058	4.99	17.6	After subtotal thyroidectomy	
	April 28 1928	51.9	1.53	-2.3	3335	64.2	2180	4.38	18.6		
	June 15 1928	52.7	1.54	-3.2	3590	68.1	2330	4.02	17.3		
	November 15 1929	52.8	1.54	+1.2	3215	60.9	2088	4.76	18.2		
	March 28 1929	53.1	1.52	+6.8	3325	62.6	2190	5.28	18.5	On admission	
10	April 11 1929	53.0	1.52	+2.2	3270	61.7	2150	5.17	17.8	After a course of Lugol's solution	
	April 30 1929	53.9	1.53	+4	2960	55.0	1936	4.53	18.4	After subtotal thyroidectomy	
	September 6 1929	61.4	1.61	-2.4	3405	55.4	2114	4.53	19.2	Before subtotal thyroidectomy	
	July 4 1929	47.0	1.44	+4.3	3250	69.2	2258	4.38	14.7	After subtotal thyroidectomy	
11	December 11 1929	60.4	1.60	-20	3260	53.0	2060	4.43	18.0	On admission	
	March 6 1930	47.4	1.45	+7.4	4500	95.0	3100	4.44	12.8	After subtotal thyroidectomy	
	March 19 1930	46.4	1.43	+3.5	3620	78.0	2530	5.11	13.6	Before subtotal thyroidectomy	
12	April 3 1930	46.5	1.43	+5	3380	72.7	2365	4.57	14.1	After subtotal thyroidectomy	
	April 25 1930	48.7	1.46	-1	3430	70.5	2350	4.46	15.6	On admission	
	February 25 1930	62.9	1.74	+5.0	5100	81.0	2930	4.88	14.7	After subtotal thyroidectomy	
	March 21 1930	60.3	1.71	-9	4555	75.5	2663	4.90	18.0	Before subtotal thyroidectomy	
13	April 3 1928	42.1	1.28	+4.0	3285	78.0	2570	4.68	16.2	After subtotal thyroidectomy	
	April 11 1928	42.0	1.28	-3	3245	77.3	2535	4.15	16.5	Before subtotal thyroidectomy	
	May 4 1928	43.3	1.31	-1	3200	73.9	2450	4.08	16.3	After subtotal thyroidectomy	
	May 6 1930	48.0	1.48	+109	3965	82.6	2680	5.09	16.5	On admission	
19	May 16 1930	46.6	1.45	+7.4	3320	71.2	2290	5.09	15.9	After a course of Lugol's solution	
	June 4 1930	49.2	1.49	+32	3600	73.2	2410	4.79	15.1	After subtotal thyroidectomy	
	November 11 1930	56.7	1.57	+30	3500	61.8	2230	4.12	15.0	After subtotal thyroidectomy	
									39.8		

TABLE 4  
*Comparison of the percentage decreases of the blood volume and the basal metabolic rate after subtotal thyroidectomy*

Case number	Before thyroidectomy				After thyroidectomy				Percentage decrease			
	Basal metabolic rate	Blood volume			Basal metabolic rate	Blood volume			Basal metabolic rate	Blood volume		
		Total	Per kilogram	Per square meter		Total	Per kilogram	Per square meter		Total	Per kilogram	Per square meter
1	155	3730	81.0	2590	90	3295	59.5	2125	41.9	11.9	26.5	21.9
2	131	3315	73.0	2240	85	2875	61.8	1930	35.1	13.3	15.3	13.8
3	151	3610	58.2	2099	102	3190	50.0	1823	32.5	11.6	14.1	13.5
4	134	5120	76.4	2910	94	4320	63.4	2440	29.9	15.6	17.0	16.2
5	173	4041	74.1	2574	107	3590	64.1	2272	38.2	11.2	13.5	11.7
6	133	3970	66.9	2480	86	3595	65.0	2300	35.3	9.4	2.8	7.3
7	130	4880	69.0	2638	84	4120	57.9	2215	35.4	15.6	16.1	16.0
8	147	3100	62.4	2095	85	2875	56.8	1843	42.2	7.3	0.0	12.0
9	155	4522	86.3	2958	80	3170	61.0	2058	48.4	30.4	29.3	30.4
10	168	3325	62.6	2190	194	2960	55.0	1936	38.1	11.0	12.1	11.6
11	143	3250	69.2	2258	80	3200	53.0	2000	44.0	1.5	23.4	11.4
12	174	4500	95.0	3100	105	3380	72.7	2365	39.7	24.9	23.5	23.7
13	150	5100	81.0	2930	91	4555	75.5	2663	39.3	10.7	6.8	8.9
14	140	3285	78.0	2570	97	3245	77.3	2535	30.7	1.2	0.9	1.4
19	209	3965	82.6	2680	132	3600	73.2	2410	36.8	9.2	11.3	10.1

along with the fall in metabolism. Following the partial removal of the thyroid gland there was in these patients a further decrease in the circulating blood.

#### DISCUSSION

The results given above clearly illustrate the adjustment of circulation in time of need. The elevation of metabolism in thyrotoxicosis places the patient at rest on a physiological level with a normal person during physical exertion. The increased demand for oxygen necessitates an increase in the circulation. The relation between the basal metabolism and the pulse rate is a well recognized fact in Graves' disease (18). The behavior of the cardiac output and the blood flow under the same circumstances has already been referred to. Barcroft's classical experiment (19) on the exteriorized spleen has shown that there is an outpouring of blood from this important reservoir during exercise. This sudden addition of blood to the systemic circulation must mean a considerable increase in its volume. As hyperthyroidism and physical exercise are closely analogous, the increase in the circulating volume in these two conditions may be looked upon as the same physiological and compensatory mechanism. The extra load thus imposed on the heart may well serve to throw light upon the pathogenesis of heart failure in thyroid disease. To push the analogy further there seems little distinction, etiologically, between the thyroid heart and the heart disease occasionally observed among the athletes, the difference being that in the case of the former the insult to the cardiovascular system is constantly operative and the damage becomes, therefore, severer. The lowering of metabolism after partial thyroidectomy means a decrease in oxygen requirement and consequently there is a return of the blood to its normal volume. Similarly in hypothyroidism, in which an abnormally low metabolism prevails, the opposite change in blood volume would be expected to take place. This is actually the case, as was demonstrated by Thompson (20). This author employed the dye method for the determination of blood volume. He was able to increase the plasma volume of his patients, frequently to the extent of 25 per cent, by the administration of thyroxin or thyroid extract. On omission of the glandular therapy the plasma volume invariably decreased again. Table 5 records the findings by the carbon monoxide method for blood volume in a case of

TABLE 5  
*The blood volume and basal metabolic rate in a case of myxedema, during the course of treatment duration*

Date	Weight	Body surface	Basal metabolic rate	Blood volume			Red blood cells	Oxygen capacity	Cell volume	Remarks
				Total	Per kilogram	Per square meter				
	kgm	square meter	per cent	cc	cc	cc	millions	volmes per cent	per cent	
January 21, 1929	81.5	1.85	-27	3960	48.6	2140	4.47	15.9		On admission
February 6, 1929	76.6	1.80	+ 1	4230	55.2	2350	4.22	13.9		After 3.36 grams of dried thyroid
March 2, 1929	74.7	1.78	- 9	4300	57.6	2415	4.29	13.6	33.1	After 1.80 gram more of dried thyroid
March 18, 1929	73.8	1.77	- 3	4760	64.5	2690	4.35	15.2	31.0	After 2.04 grams more of dried thyroid

myxedema, these are entirely in accord with Thompson's observations.

We may now turn to consider the question from another standpoint, namely, that of hemodynamics. In hyperthyroidism, particularly in exophthalmic goiter, the blood supply of the thyroid gland is considerably increased. The pulsation and bruit often elicited over the diseased gland indicate a marked increment of its vascularity. The neck veins and the carotid arteries are frequently dilated. There is thus a wide capillary bed in the neck which may short circuit a large portion of the blood and may bear a close resemblance to an arteriovenous fistula. The similarity between the two conditions may be exemplified further. In hyperthyroidism a high pulse pressure is quite a constant feature. There is flushing of the skin from which may be inferred a diminution of the peripheral resistance. There seems an increase in the vascular bed generally as well as over the thyroid gland. This is precisely what occurs in the arteriovenous fistula. Holman (21) in a series of experiments has shown that in the case of an arteriovenous fistula, the gradually increasing dilatation of the vascular bed is compensated by a corresponding increase in the blood volume and that this volume change may be easily rectified by either temporary or permanent closure of the fistula. In the light of Holman's findings it seems difficult to expect the circulatory volume to behave otherwise in exophthalmic goiter. The partial excision of the hypertrophied thyroid may be rightly compared with the closing of the arteriovenous fistula. In this connection it is interesting to note that in Case 14 of the series here reported, (the case of toxic adenoma) in which no profound vascular change of the thyroid gland was present the initial blood volume did not appear excessively high nor was a postoperative reduction observed.

From these theoretical considerations and the actual experimental data it seems highly plausible to explain the pathologic physiology of the circulatory system in thyrotoxicosis on the following basis. The elevated metabolism means increased oxygen consumption and increased transportation of metabolites. This increased demand must be met by some adjustment on the part of the circulating medium. Such an adjustment is accomplished by an elevation of the pulse rate, augmentation of the cardiac output, acceleration of the blood flow,

enlargement of the vascular bed and by increase of the blood volume. The great vascularity of the diseased thyroid contributes to aggravate all these changes. The resulting extra load on and insult to the heart must be considerable and, when long continued, must eventually produce fatigue and failure. It is thus not necessary to assume any direct toxic action of the thyroid hormone on the heart in order to seek an understanding of the familiar cardiovascular disturbance in hyperthyroidism.

#### SUMMARY

The circulating blood volume of 21 cases of hyperthyroidism was determined by the carbon monoxide method and was found to be much higher than the average normal volume. Of the 15 cases in which subsequent determinations were possible following subtotal thyroidectomy all but one (a case of toxic adenoma) showed a definite reduction of the blood volume after the operation. Five cases were studied after the administration of Lugol's solution. This treatment alone had an appreciable effect in decreasing the blood volume along with the fall in the basal metabolic rate.

The significance of these findings and their bearing on the pathogenesis of the cardiovascular disturbance in thyroid disease are discussed.

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## THE ACID BASE EQUILIBRIUM IN PATHOLOGICAL CONDITIONS

### II ALKALOSIS OBSERVED IN HYPERTENSIVE STATES<sup>1</sup>

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Nephritis with nitrogen retention has generally been considered to be associated with a progressive acidosis. Whitney (1) and Chace and Myers (2) were among the first to point this out. The latter workers observed that all fatal cases of chronic nephritis with marked nitrogen retention showed a severe acidosis, sufficient in many instances to be the actual cause of death. Since acidosis is a term applied to conditions in which the bicarbonate concentration of the blood is low it may arise from several causes: a reduction of the total base concentration or an increase of the acid ions. An influx of acid into the blood may occur from an abnormal formation as in diabetes mellitus or from a decreased elimination as in chronic nephritis. Marriott and Howland (3) demonstrated an increase in the phosphate of the serum in nephritis with a lowered CO<sub>2</sub> capacity of the plasma and decrease in plasma pH. Greenwald (4), Denis and Minot (5), Salvesen and Linder (6), and Schmitz Rohdenburg and Myers (7)

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Most of the cases in this study were on the service of one of us (C. T. W.) in the Medical Wards of St. Luke's Hospital; the rest were in the Medical Wards of the City Hospital. We are indebted to Drs. R. W. Scott, W. F. Burger and H. A. Ruprecht for the privilege of studying the cases at City Hospital.

have also shown retention of acid phosphate in nephritis. De Weaselow (8) pointed out that on the average the retentions of phosphates and urea run a parallel course. That phosphate may not be the only acid constituent to be retained was pointed out by Denis and Hobson (9), Loeb and Benedict (10), and Wakefield (11), who showed that in nephritis with nitrogen retention the  $\text{SO}_4$  ion is retained in the blood and roughly parallels the nitrogen retention.

Salvesen (12) studied the changes of the individual cations and anions along with the pH and  $\text{CO}_2$  tension in the blood of 37 cases having renal disorder. It was found that an acidosis is very frequent, and if acute nephritis is excluded the condition for acidosis is kidney insufficiency. In two of the cases observed by Salvesen the acidosis was attributable to an increased chloride. It was concluded that the most important factor causing acidosis is an accumulation of organic acid in the blood and this factor is aided by a more or less pronounced loss of base. Phosphate retention was considered of no importance.

Recently Peters, Wakeman, Eisenman and Lee (13) have also considered the factors causing the acidosis of nephritis by making a total acid-base equilibrium study of the plasma. The most striking fact observed was the frequency of reduction of the bicarbonate concentration. One of the electrolyte disturbances most frequently encountered was a deficiency of the total electrolyte (base) concentration of the serum and this was considered instrumental in the production of acidosis in a rather large proportion of cases. Phosphate increases were found relatively insignificant factors in causing acidosis. It was also concluded that sulfate and organic acids are less important than had been supposed. Chloride as well as bicarbonate was diminished in most cases with deficient base, and a reduction of Cl and base was usually encountered when vomiting was a serious symptom or when the patients had received limited amounts of salt and excreted large amounts of fluid. It was pointed out that one rather surprising fact seems to have been accepted without comment by previous observers, "that bicarbonate is never found elevated in a condition in which vomiting is such a prominent symptom."

Before the appearance of the above report, the possibility of finding an increased bicarbonate in connection with cases showing vomiting had attracted us to study cases of hypertension in which vomiting

was a prominent symptom since it was early pointed out by Kast, Myers and Schmitz (14) that vomiting may lead to alkalosis. A study of the acid base equilibrium has been made in twelve cases, which were characterized by a persistent high blood pressure with only moderate or no nitrogen retention. The first two cases reported were the only ones in which there was considerable vomiting.

#### METHODS EMPLOYED

The blood, usually 25 or 30 cc., was withdrawn from an arm vein under oil and without stasis. All samples were taken before breakfast. The blood was immediately transferred under oil to a special centrifuge tube and tightly stoppered. After removing enough blood under oil for oxygen capacity determinations the sample was centrifuged and the plasma separated as quickly as possible and placed under oil in a special tube as described elsewhere (15). The CO<sub>2</sub> content and pH estimations were then made immediately.

To prevent clotting, heparin was employed (1 mgm. heparin for 5 cc. of blood). This was carefully weighed and placed in the centrifuge tube. A small drop of distilled water was added to the heparin in order to make a paste. This made possible the free mixing of the blood with the heparin when placed under oil.

The carbon dioxide content was determined by the method of Van Slyke and Neill (16). For the estimation of chlorides the method of Van Slyke (17) was employed. With the introduction of the modification by Wilson and Ball (18) the latter procedure was employed. The pH was determined colorimetrically as described by Myers and Muntwyler (15). The procedure was the same except in the adjusting of the saline indicator solution. Instead of employing 1 per cent NaOH to adjust the saline solution to pH 7.55, CO<sub>2</sub> free air was drawn through the solution until the pH was 7.4 to 7.5. For total base, the method of Stadie and Ross (19) was employed. The phosphate of the plasma was not determined in all cases. For this reason the total base figures were not corrected for the loss by combination with phosphate. Since however the phosphate was found normal when determined, only a small error is introduced by this omission. For the determination of total protein 0.5 cc. of plasma was subjected to a Kjeldahl procedure. From the total nitrogen 30 mgm. per 100 cc. was sub-

tracted This is to correct for nonprotein nitrogen, as the latter was not determined in all cases

#### CALCULATIONS

The value for grams total protein per 100 cc was converted into milliequivalents of base combined with protein by the formula of Van Slyke, Hastings, Hiller and Sendroy in the form used by Peters, et al (13),

$$BP = 1072 P(pH - 5.04),$$

in which an albumin globulin ratio of 1.8 was assumed BP and P represent respectively the milliequivalents of base combined with protein per liter of serum and grams of protein per 100 cc

The milliequivalents of base combined as  $BHCO_3$  were obtained from the pH and total  $CO_2$  by the following equation

$$m\ Eq\ BHCO_3 = CO_2 - \frac{CO_2}{(10^{pH-6.10} + 1)},$$

in which  $CO_2$  is the mM concentration of total  $CO_2$

The total measured acid (TA) in this discussion is taken as

$$TA = BHCO_3 + BCl + BP,$$

where  $BHCO_3$ ,  $BCl$  and  $BP$  represent milliequivalents of base combined with  $HCO_3$ , Cl and P respectively The difference between the total base (TB) determined and the total measured acid is termed the undetermined acid and represents the amount of base equivalent to the phosphate, sulfate and salts of organic acids

#### RESULTS

The results of the plasma analyses of twelve cases with persistent hypertension are collected in Table I Whenever possible, the cases were observed over considerable periods of time, the blood samples being taken either every week or every other week In this way successive changes to termination were followed

If one assumes a plasma bicarbonate concentration of 31 m Eq as the upper limit of normal, a condition of alkalosis existed in six cases at some period in the observation (Cases 1, 2, 3, 4, 5 and 7) The highest plasma bicarbonate was observed with Case 1, where the

concentration varied between 34 and 37 m Eq over a period of twenty two days just preceding death, and during which time the patient presented an uncontrollable vomiting. The only other case to show vomiting was Case 2 and here the plasma bicarbonate reached a maximum value of 35 m Eq. The lowest bicarbonate concentration encountered was 18.1 m Eq. This was found in the terminal blood of Case 3. It should be pointed out however that this case had previously shown a maximum bicarbonate concentration of 32 m Eq. The great majority of the plasma bicarbonate concentrations were above 27 m Eq.

The colorimetric pH of the plasma was found elevated in a greater number of cases than was the bicarbonate. A pH above 7.48 was observed in all but one case the maximum of 7.59 being observed with Case 4. The lowest pH value 7.28, was obtained in the terminal blood of Case 3.

Considerable variation was encountered in the total base and chloride concentrations. Cases 1 and 2 which were accompanied by vomiting had the lowest chloride concentrations observed namely 83 and 85 m Eq respectively. The highest chloride concentration observed was 107 m Eq (Case 11). With the exception of 8 and 9, all the cases had chloride concentrations below 100 m Eq at some period during observation. In the individual cases observed over considerable periods of time the chloride concentration fluctuated widely. Cases 1 and 2 were accompanied with chloride decreases of 13 and 17 m Eq respectively. This was undoubtedly augmented by the loss of chloride with vomiting. This decrease of chloride was accompanied by a decrease of total base and at the same time an increase in the bicarbonate concentration. The increase in bicarbonate was, however, not as great as the decrease in chloride with the consequence that the total measured acid concentration was decreased. Cases 3 and 5 had chloride changes of 11 and 13 m Eq respectively. The highest bicarbonate concentration of Case 3 was present with the lowest chloride concentration. This was also the observation with Case 5. The total base appeared in most instances to change in the same direction as the chloride. The total base concentration varied from a high value of 176 m Eq in Case 7 to a low value of 137 m Eq in Case 1. The total measured acid concentration was more uniform than the total base varying between 152 and 132 m Eq.

## ALKALOSIS IN HYPERTENSIVE STATES

TABLE I  
*The acid base balance in cases of persistent hypertension*

Case number	Date	Plasma						Urea nitrogen mgm per cent
		pH	HCO <sub>3</sub>	Cl	Total protein	Total measured acid	Total base	
1	September 12, 1928	7.45	28	96	<i>m</i> <i>Lq</i> 16	<i>m</i> <i>Eg</i> 140	<i>m</i> <i>Ei</i> 146	13
	February 7, 1929	7.54	30	89	17	136	140	
	February 15, 1929	7.54	37	85	17	139	140	
	February 23, 1929	7.56	36	84	16	136	140	
	March 1, 1929	7.54	34	84	17	135	140	
	March 9, 1929	7.56	36	83	16	135	137	
	September 19, 1928	7.39	26	102	14	142	156	
2	October 9, 1928	7.52	29	93	16	138	142	23
	October 24, 1928	7.41	35	85	15	135	140	
	March 29, 1929	7.42	30	105	16	151	156	
3	May 23, 1929	7.44	28	103	15	146	156	17
	June 22, 1929	7.50	31	98	15	144	157	
	June 24, 1929	7.48	32	94	15	141	151	
	July 3, 1929	7.47	32	100	15	147	156	
	July 17, 1929	7.34	29	100	15	144	157	
	September 8, 1929	7.40	28	99	18	145	153	
	October 2, 1929	7.35	21	99	17	137	160	
	October 15, 1929	7.28	18	103	15	136	166	
	February 13, 1930	7.56	31	94	16	141	142	
	February 19, 1930	7.59	32	91	17	139	140	
4	March 14, 1930	7.54	30	91	17	138	140	32
	March 29, 1929	7.48	29	97	18	144	146	
	January 3, 1930	7.47	28	101	17	146	160	
5	January 16, 1930	7.44	31	97	15	143	165	13

64 nonprotein nitrogen  
39 nonprotein nitrogen



## DISCUSSION OF RESULTS

At the outset of this study, interest was attracted to the influence of vomiting on the acid-base equilibrium of the plasma. It has been shown by a number of workers in pyloric obstruction, and by others (20) in cases of periodic vomiting from other causes, that there is an alkalosis as a result of the loss of acid from the body in the form of HCl. The question of a loss of chloride in the vomitus was considered by Peters, Wakeman and Lee (21) in connection with the reductions of chloride and base observed in nephritis. They found that the vomitus of uremic patients, although not a negligible source of chloride loss, is usually not an important one. The chloride concentration in the vomitus often exceeded that in the urine, though the total volume of the vomitus was so small in comparison with that of urine that the chloride actually lost in the urine was usually far in excess of that in the vomitus. Further, they found that vomiting in nephritis does not eliminate chloride alone, but also a considerable amount of base. This they offered as partial explanation for lack of bicarbonate excess even with the most persistent emesis. The absence of alkalosis was attributed in part, furthermore, to the tendency to lose base in the urine in combination with acids which would ordinarily be neutralized by ammonia, since it had been shown by Van Slyke, Linder, Hiller, Leiter and McIntosh (22) that there is a decreased ability in nephritis to form ammonia.

It is of interest then to consider Cases 1 and 2 in detail, since they were the only ones to show vomiting. At no time during the period of observation was alkali administered. Case 1 presented persistent vomiting from February 7 to March 1, during which time, over a period of several days at a time, very little food could be retained. Oral administration of NaCl, NH<sub>4</sub>Cl and dilute HCl was attempted from February 23 to March 9. Very little was retained as a result of the vomiting. Case 2 showed intermittent vomiting spells from the first observation until termination. Both of these cases showed a progressive fall in chloride and total base concentrations. The decrease in total base was not as great as the fall in chloride and the difference was more than made up by an increase in bicarbonate. The total measured acid concentration, however, showed a progressive decrease. A high pH was observed in both instances. Case 1 showed

a condition of uncompensated alkalosis over a period of twenty two days just preceding death. Case 2 attained a maximum plasma bicarbonate concentration of 35 m Eq. No analyses were made of the vomitus so that the amount of acid and base lost by this route is not known. It may be that in these cases with little nitrogen retention there is considerably more free HCl lost in the vomitus than in those described by Peters, Wakeman and Lee (21).

Hartman Smyth and Moser (20) found that Cl seldom appeared in the urine or only in very small amounts when the serum chloride was below 90 milliequivalents. Peters, Wakeman and Lee (21) have shown that in some cases of nephritis there is a tendency for both base and chloride to be excreted in the urine when the serum chloride has fallen below the level which in normal individuals determines a chloruria. In Case 1 (see Table II) the plasma chloride concentration was definitely below 90 m Eq., yet chloride was appearing in the urine. On the other hand there was apparently a conservation of base in that even though the bicarbonate concentration of the plasma was definitely above normal, the urine showed an acid reaction. In the other cases studied, the urine showed an acid reaction with the exception of Case 3. Case 6 had an extremely acid reaction while Case 4 had a urine acid to pH 6.0 in spite of the 31 m Eq. plasma bicarbonate concentration. It is of interest in this connection that Kast, Myers and Schmitz (14) pointed out that in certain cases alkalosis is readily produced by bicarbonate administration for the reason that the kidneys do not readily eliminate alkali. In two of their cases the urine remained strongly acid (pH 5.2 and 5.12) despite an alkalosis.

The changes in acid base balance appear in general to be such that a lowered plasma chloride accompanies the elevated bicarbonate. The elevated bicarbonate may be present in spite of a lowered total base concentration. In Cases 1, 2, 4, 5 and 10, the elevated bicarbonate was accompanied by a total base concentration below 150 m Eq. and a chloride concentration at or below 100 m Eq. It might be inferred that in these cases there were complications of heart failure with high bicarbonate as a result of anoxic anoxemia as reported by Peters, Bulger, and Eisenman (23). Cases 1 through 9 were under careful observation since they were on the service of one of us (C T W.)

TABLE II  
*Urine changes in cases of persistent hypertension*

Case number	Date	Urine		Plasma	
		pH	Chloride grams per liter	Cl <i>m Eq</i>	HCO <sub>3</sub> <i>m Eq</i>
1	February 23, 1929	6.5	1.8	84	36
	March 1, 1929	6.1	2.1	84	34
	March 9, 1929	6.5	1.1	83	36
3	March 29, 1929	7.4	4.8	105	30
	April 1, 1929	7.5	4.2	105	30
	April 4, 1929	7.0		103	28
	April 6, 1929	7.2		98	31
	May 23, 1929				
	June 22, 1929				
4	February 13, 1930	5.7	4.4	94	31
	February 15, 1930	5.9	5.4	91	30
	March 14, 1930				
	March 20, 1930				
5	March 29, 1929	5.4	5.4	97	29
	April 1, 1929	6.5	1.6	101	28
	January 3, 1930	6.3	4.2	97	31
	February 10, 1930	5.7	4.3	98	30
6	January 16, 1930	5.0	1.9	100	29
	January 31, 1930	4.7	2.0	101	30
	February 10, 1930	4.9	2.0	103	31
	February 11, 1930	5.0	3.8		
	February 15, 1930	4.4	3.4	104	27

in the Medical Wards of St Luke's Hospital Cardiac insufficiency was in evidence in Cases 3, 4, 5 and 6 at some time during the period of observation It should be pointed out, however, that no cardiac insufficiency was present in Cases 1, 2 and 7 where the highest plasma bicarbonate concentrations were observed Further, in Case 3, during the early period of observation when an alkalosis was manifested, cardiac insufficiency was not a pronounced factor During the later period of observation a very considerable degree of edema developed and myocardial insufficiency was in evidence, but at this time the

bicarbonate concentration had fallen to such a degree that an acidosis existed.

Salvesen (12) found that an acidosis is of frequent occurrence in diseases of renal origin, whereas an alkalosis is very seldom if ever met with. However, he pointed out that a change in the pH to the alkaline side, due probably to a low  $\text{CO}_2$  pressure, may be seen more frequently. Unusual clinical interest is attached to Cases 1, 2, 4 and 12 of this study. The pictures they presented would have been regarded as typical examples of acidosis had not the determinations of the plasma  $\text{CO}_2$  content revealed the presence of an alkalosis. Attention has frequently been called to the marked similarity of the clinical pictures of these two disturbances of the acid base balance and the frequent necessity for chemical blood studies for their differentiation. Therapeutic efforts to control the alkalosis associated with hypertension have been of little if any, value in averting the fatal issue. Where chloride depletion is the result of vomiting the administration of chloride would seem to be indicated. Oral administration of chloride was attempted with Case 1. Little of value was accomplished due to the interference from the vomiting. Intravenous administration of chloride would probably have given some relief.

#### SUMMARY

1 A study of the acid base equilibrium has been made in twelve cases showing persistent hypertension with only slight nitrogen retention.

2 A plasma bicarbonate content above 31 m Eq was observed in six cases while an elevation of plasma pH to above 7.48 was found in all but one case.

3 Two cases presented severe vomiting which was accompanied by a loss of chloride and total base from the blood and an elevation of bicarbonate.

4 Chloride and total base concentrations were very irregular. The chloride concentration in the majority of cases was below 100 m Eq. In several cases a high bicarbonate was in evidence even in the presence of a low total base concentration.

5 It is logical to expect that if vomiting occurs in cases having a tendency to an elevated bicarbonate and a lowered chloride concentration, a condition of alkalosis will result.

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#### CASE HISTORIES

**Case 1** 70812 T W female aged 38, housewife was observed over approximately two and one half years. Her chief complaints were headaches accompanied by nausea and vomiting throbbing in the head numbness and tingling in extremities progressive weight loss and nocturia During the latter period of her illness vision was seriously impaired

The salient physical and laboratory findings were slight cardiac enlargement, systolic blood pressure which varied from 180 to 260 and diastolic pressure from 130 to 160 fundi of both eyes showed arteriosclerotic changes the uterus was about the size of a grapefruit firm and apparently fibroid in nature urine showed a trace of protein during the last year of observation with occasional hyaline and granular casts serological studies of both blood and spinal fluid were negative complete gastro-intestinal x ray findings were negative

During the latter part of this patient's illness her most distressing problems were headache and vomiting from which it was almost impossible to afford any effective relief consequently weight loss was very marked During the last week of life typical carpopedal spasm was observed A period of stupor of about two days duration preceded death

Clinical Diagnosis, Malignant hypertension cardiac hypertrophy

**Case 2** 69473 J A W male aged 24, bookkeeper was first seen July 17 1928 at which time his chief complaints were headaches periodic in nature, occasionally accompanied by nausea and vomiting slight swelling of both ankles spots before the eyes and nocturia two or three times a night

History revealed a severe scarlet fever four years previous, several attacks of acute tonsillitis, appendectomy in 1918 and tonsillectomy in 1922.

A period of hospitalization at this time resulted in the following findings: albuminuric retinitis, slight cardiac enlargement, slight edema of both ankles, systolic blood pressure varied between 232 and 190 and diastolic varied from 160 to 120. Modified Mosenthal test showed a practically fixed specific gravity at a low level. Variations in day specimens were between 1.010 and 1.014. The night quantity from 8 P.M. to 8 A.M. showed a volume of 995 cc and a specific gravity of 1.010. Urine analysis showed a trace of protein and numerous hyaline and finely granular casts, a few erythrocytes and a few leucocytes.

On August 16, 1928, approximately a month after first examination, after a very severe headache, patient was taken with convulsions and for three days remained in a stuporous state. Blood pressure was 200 systolic, 120 diastolic at this time. With usual therapeutic measures, including venesection, he gradually improved and was able to leave the hospital in three weeks. During this time the highest blood urea nitrogen observed was 28.5 mgm and creatinine, 4.1 mgm. Phenolsulphonphthalein excretion was 32 per cent in two hours.

The final acid-base study, made October 24, 1928, was just two days before death. Patient became progressively weaker and died October 26, 1928, in coma.

Clinical Diagnosis Chronic glomerular nephritis, cardiac hypertrophy, slight.

*Case 8 78623* J.H., female, aged 58, housewife, complained of an illness of five years' duration, characterized by throbbing in the head, shortness of breath, nocturia, headaches and progressive weight loss. Periods of dyspnea at night of considerable severity would occur, accompanied by pain in the precordial region and in both shoulders and back. Blood pressure varied between 220 and 166 systolic and between 120 and 140 diastolic. This patient was under observation for about two years. During the early part of this period there was slight swelling of the ankles on numerous occasions. About one month before death a generalized edema developed which continued to grow worse until death. The urine constantly showed a trace to a moderate amount of protein with numerous hyaline and granular casts and was always of a low specific gravity, between 1.008 and 1.012 on numerous examinations. There was a progressive anemia as evidenced by oxygen capacity studies of the blood, which varied from 15 volumes per cent at the beginning of the study down to 9.4 volumes per cent at the time of the last analysis. Electrocardiographic study of the heart revealed a partial bundle branch block. Eye ground examination showed a marked degree of arteriosclerotic change involving both fundi. A period of coma of five days' duration preceded death.

Clinical Diagnosis Hypertension with evidence of kidney insufficiency,

generalized arteriosclerosis, partial bundle branch heart block, cardiac hypertrophy.

*Case 4* 82880 M H female, white aged 46 housewife Present illness was of more than five years duration during which main symptoms have been severe and frequent headaches frequency of urination day and night and weight loss Just before entering hospital she lapsed into a state of coma.

Physical examination revealed a well developed but poorly nourished woman in a state of coma, exhibiting Cheyne Stokes respiration Positive findings were cardiac enlargement to left anterior axillary line, systolic murmur audible over entire precordium, pulse rate 100 and regular liver palpable three fingers breadth below costal margin and slight edema of both ankles Palpable arteries were sclerotic Blood pressure ranged between 270 and 228 systolic and 160 to 150 diastolic. Eye grounds showed marked sclerosis of retinal vessels Blood nonprotein nitrogen was 30.4 and 31.8 Urine showed a moderate amount of protein a few leucocytes and occasional hyaline and granular casts The last observation reported in Table I was just two weeks before death occurred When the blood samples were taken signs of cardiac failure were not present to an appreciable degree.

*Final Pathological Diagnosis* Arteriolosclerosis of spleen, pancreas adrenals and kidneys marked arteriolosclerotic atrophy of kidneys with multiple small anemic and hemorrhagic infarctions hypertrophy and dilatation of heart very marked multiple small anemic infarctions of myocardium multiple mural thrombi of both ventricles and right auricle multiple hemorrhagic infarctions of both lungs acute interstitial pancreatitis with discrete areas of necrosis slight chronic passive congestion of liver, spleen and small intestine senile emphysema of lungs, very marked intense congestion of trigone and first portion of urethra with submucous hemorrhages thrombosis of ovarian veins emaciation senile atrophy of ovaries anthracosis of lungs slight moderate intimal fatty infiltration of the aorta and coronary arteries mild healed aortic endocarditis diverticula of bladder two in number a small cavernous angioma of liver slight melanosis coli

*Case 5* 74650 A. K. female, white aged 77 housewife Main complaints were headaches shortness of breath dizziness frequency of urination day and night, and pain in substernal region Physical examination revealed an undernourished elderly woman, many carious teeth, moderate cardiac enlargement, marked sclerosis of all accessible arteries, and a blood pressure which varied between 225 and 170 systolic and 100 to 130 diastolic Urine analysis revealed a trace of protein, and on a few occasions a positive sugar Blood urea nitrogen was never observed above 16.1 mgm per 100 cc Blood sugar values were moderately elevated during latter part of illness the highest value observed being 273 mgm Electro

cardiographic study showed evidence of myocardial involvement and left ventricular preponderance Before death a marked degree of edema developed

Final Pathological Diagnosis Bronchopneumonia, slight, edema and congestion of lungs, diffuse hemorrhagic infarctions of lower lobe of right lung, with embolism of the corresponding artery, slight hyperplasia of splenic pulp, hydrothorax, bilateral, hypertrophy and dilatation of left ventricle, moderate arteriosclerosis of coronary arteries, recent anemic infarctions of papillary muscle of left ventricle, severe arteriosclerosis of aorta, especially at the lumbar portion, marked arteriosclerosis of aortic branches, especially of the iliacs, angiosclerosis of kidneys, slight, angiosclerosis of pancreas, marked, chronic interstitial pancreatitis, moderate, with old focal fat necrosis, edema of right hand, right leg, and external genitalia, anemia, emaciation, old firm adhesion of omentum to left Fallopian tube, old adhesions about gallbladder, adenoma of right adrenal, senile emphysema of lungs, senile atrophy of liver, spleen, adrenals, internal genitalia, and breast Nodular hyperplasia of splenic pulp and marked angiosclerosis of spleen

*Case 6 80440 S O*, female, aged 66, white, housewife Present illness of seven years' duration, the main symptoms being headaches, dyspnea, palpitation, dizziness and nocturia Physical examination showed a moderately obese female, with moderate cardiac enlargement, and no demonstrable edema Blood pressure varied during 40 days' observation from 220 to 190 systolic and from 128 to 104 diastolic Eye grounds showed arteriosclerotic retinitis Leucocytes, 8800, hemoglobin, 85 per cent, phthalein test, 10 per cent the first hour and 25 per cent the second hour Variations in specific gravity of 2 hour day specimens of urine were from 1.004 to 1.008 Volume of urine, 8.00 P M to 8.00 A M , was 1005 cc with a specific gravity of 1.018 Blood chemical studies failed to show any degree of nitrogen retention Subject is still living

Clinical Diagnosis Hypertension with evidence of kidney insufficiency, cardiac hypertrophy

*Case 7 76444 L D*, female, aged 56, white, housewife Present illness was of one year's duration, onset occurred with numbness and loss of function of left side of body, which recovered with bed rest A similar accident occurred one year later Headache, dizziness and nocturia were constantly experienced throughout illness Blood pressure was systolic 198, diastolic 106 Urinalysis showed no protein, but an occasional hyaline cast Blood count red blood cells, 4,270,000, leucocytes, 7400, hemoglobin, 78 per cent Blood chemistry blood sugar, 91 mgm , and urea nitrogen, 11.9 mgm Patient is still living

Clinical Diagnosis Cerebral hemorrhage, generalized arteriosclerosis, hypertension

*Case 8 E B*, female, aged 53, white, housewife Illness was of five

years duration characterized by headaches constipation, dizziness tingling of fingers and toes nocturia and for the last year failing vision

Chief physical findings were obesity, edema beneath both eyes moderate cardiac enlargement, blood pressure systolic 250, diastolic 140 Urine showed trace of protein, hyaline and granular casts Patient refused to enter hospital for more detailed study consequently complete findings are not available

Clinical Diagnosis Hypertension malignant, cardiac hypertrophy

*Case 9 K 1225* T H female aged 60, white, housewife Present illness was of four years duration during which time three mild apoplectic strokes occurred Numbness and tingling of fingers and toes nocturia and occasional headaches with vertigo have been experienced

Physical examination revealed a slightly undernourished white female There was slight left facial asymmetry with paralysis of whole left side of body Heart was moderately enlarged to the left with accentuated aortic second sound Accessible vessels were markedly sclerotic Eye grounds examination showed arteriosclerotic retinitis Blood pressure varied from 230 to 180 systolic and 130 to 95 diastolic Red blood cells 4 300 000 leucocytes 8200 hemoglobin 80 per cent Urine showed a trace of protein hyaline and granular casts Blood Wassermann was negative Spinal fluid cell count, 4 per c.mm globulin negative Blood urea nitrogen 12.4 and 13.4 mgm per 100 cc. on two examinations

Clinical Diagnosis Arteriosclerosis general, hypertension cerebral hemorrhage.

*Case 10 82588* C D, female aged 61 white, housewife Present illness was of about ten years duration characterized by headaches dizziness and nocturia Bed rest on a number of occasions for varying periods resulted in subjective improvement Salient features of physical examination were obesity slight cardiac enlargement, obese and pendulous abdomen no edema moderate cardiac enlargement Blood pressure varied from 186 to 256 systolic and 110 to 160 diastolic Red blood cells 4 540 000 leucocytes 8100 hemoglobin 90 per cent Mosenthal test showed a fixation of specific gravity at a low level the greatest variation being between 1.005 and 1.012 Night volume was 112 cc. with specific gravity 1.007 Eye grounds showed arteriosclerotic retinitis Urinalysis revealed protein in moderate amount hyaline and granular casts

Clinical Diagnosis Malignant hypertension with evidence of kidney insufficiency cardiac hypertrophy

*Case 11 77120* J S male, aged 63 white Onset of present illness was marked by a paralytic stroke involving left side of body one year ago with recovery There had been swelling of feet and ankles and dyspnea for one year A second stroke involving left side occurred four months ago A third stroke affecting the left side of the body and the speech center led to present hospitalization

Physical examination showed fixation of right pupil, left side of mouth droops, cardiac enlargement, liver not palpable, pitting edema of both legs, knee jerks hyperactive Blood pressure systolic 210, diastolic 130 Leucocytes, 5850, hemoglobin, 70 per cent Urine acid, trace of protein, no casts Wassermann, negative Blood urea nitrogen, 20 mgm per 100 cc, blood glucose, 100 mgm per 100 cc

Clinical Diagnosis Diffuse hyperplastic arteriosclerosis, cardiac hypertrophy, right cerebral hemorrhage

*Case 12* 82884 M C, female, aged 48, white, housewife Complaint was of ten years' duration, characterized by headaches, dizziness and nocturia Past history included numerous infected teeth and acute appendicitis, which was followed by fecal fistula which later healed Physical examination showed marked obesity, no demonstrable cardiac enlargement, soft systolic murmur over aortic area Blood pressure varied during three day period of observation from 230 to 186 systolic and from 130 to 90 diastolic Eye grounds showed sclerosis of retinal vessels

Blood urea nitrogen varied from 25.7 mgm on admission to 67.5 before death Blood sugar varied between 113 and 170 mgm, uric acid from 4.4 to 7.4, nonprotein nitrogen from 50 to 132, and creatinine from 6.2 to 11.3 mgm Red blood cells, 4,800,000, leucocytes, 8000, hemoglobin, 90 per cent Urinalysis showed trace of protein and a few hyaline casts Death occurred in a state of uremia with a complicating hypostatic pneumonia

Clinical Diagnosis Hypertension with evidence of kidney insufficiency, uremia and hypostatic pneumonia

## A STUDY OF GLYCOLYSIS

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### INTRODUCTION

Since Claude Bernard (1) first noted the disappearance of sugar from drawn blood in 1856 this phenomenon has been the subject of much interest and investigation. Following the discovery of insulin, studies on glycolysis were made with renewed interest in the hope that experiments *in vitro* might shed some light upon the rôle of insulin in carbohydrate metabolism. The rate of disappearance of sugar from blood *in vitro* and the rate of glycolysis are commonly considered to be synonymous, a custom which will be followed in this discussion. The accumulated data on glycolysis show a very striking lack of uniformity. The purpose of this report is to present a series of observations on glycolysis and to discuss some of the principal factors influencing the rate and the amount of sugar glycolyzed.

### METHOD

Patients in the University Hospital served as subjects for the following experiments. They were unselected except that no patient with an abnormally high nonprotein nitrogen content of the blood was used. From each patient 10 cc. of blood was drawn two or two and one half hours after a meal. This blood with the exception of one half cc. for the determination of the initial sugar content, was transferred immediately to a sterile 50 cc. Erlenmeyer flask containing a bent paper clip. The flask was loosely stoppered with sterile cotton, shaken for fifteen minutes to defibrinate the blood and incubated in a moist chamber at 37° C. The blood was well shaken before and after each of the sugar determinations, which were made at two hour intervals according to the method of Gibson, Mitchell and Larimer (2).

TABLE I  
*Total sugar glycolyzed in a series of bloods*

Case	Diagnosis	Sex	Age	Total sugar glycolyzed Hours				
				2	4	6	8	10
				mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc
1	Myelogenous leukemia	M	59	98	128*			
2	Myelogenous leukemia	M	48	94	158*			
3	Diabetes mellitus	M	50	93	103	166	179	223*
4	Myelogenous leukemia	F	59	87	118*			
5	Myelogenous leukemia	F	41	81	148	186*		
6	Congenital heart disease	M	30	80	112*			
7	Diabetes mellitus	M	49	75	143	215		242
8	Chronic endocarditis, cardiac decompensation, osteoarthritis	M	57	71	108	132*		
9	Multiple fractures, empyema	M	56	69	129	149*		
10	Gastric neurosis	M	51	68	100	128*		
11	Malignant lymphomata, Hodgkin's type	F	20	67	128*			
12	Arteriosclerosis, hypertrophy of prostate	M	57	63	119	162*		
13	Banti's disease, parotitis epidemic	F	22	63	110*			
14	Septicopyemia	F	27	57	97	136*		
15	Arsenical hepatitis	M	30	53	93	133		
16	Hysteria	F	26	53	76	103*		
17	Chronic myocarditis, hypertension	M	47	50	90	143		
18	Mixed tumor of parotid gland with generalized metastases	M	18	50	99	133		
19	Psychoneurosis	M	39	50	100	140	175*	
20	Pulmonary tuberculosis, tubercular peritonitis	M	31	50	103			
21	Mitral stenosis	M	43	49	87	117*		
22	Arsenical hepatitis	M	23	48	95	130*		
23	Peptic ulcer	F	30	48	83	127*		
24	Diabetes mellitus	M	27	47	86	127*		
25	Pernicious anemia	F	45	47	77	110*		
26	Chronic progressive vascular nephritis, chronic uremia	M	60	46	91			
27	Exophthalmic goiter	F	41	44	82	121*		
28	Diabetes mellitus, adenoma of thyroid	M	54	44	96			

TABLE I—Continued

Case	Diagnosis	Sex	Age	Total sugar glycolized: Hours				
				2	4	6	8	10
29	Syphilitic aortitis aortic insufficiency	M	62	44	98	140*		
30	Chronic myocarditis chronic rheumatic endocarditis latent syphilis	F	34	43	72	96*		
31	Chronic cholecystitis	M	30	43	87	120*		
32	Bronchopneumonia	M	53	42	82	120*		
33	Arteriosclerosis gangrene of toe	M	50	42	83		149*	
34	Cardiac decompensation	M	61	42	102	132*		
35	Malignant lymphomata Hodgkin's type	M	43	41	69	100*		
36	Pernicious anemia	F	59	40	74	93	149*	
37	Diabetes mellitus	M	15	36	128	172	232	
38	Chronic rheumatic endocarditis	M	58	36	72		129*	
39	Chronic interstitial nephritis	M	42	35	77	146	164	203*
40	Tuberculosis	M	53	35	63	105*		
41	Arteriosclerosis	M	71	34	71	97	145*	
42	Visceroptosis	M	24	34	94	114		
43	Pernicious anemia	M	68	34	82*			
44	Lymphocytic leukemia	M	20	34	57	95*		
45	Cardiospasm	M	59	33	78			
46	Hypertension cardiac decompensation	M	64	33	58	90*		
47	Catarrhal jaundice	M	39	33	80	114*		
48	Subacute nephritis	M	34	32	66	119	175*	
49	Cardiac decompensation	M	43	32	89	148	172*	
50	Diabetes mellitus gangrene of toe	M	57	31	72	107	175	232
51	Multiple leiomyomata of uterus	F	28	31	78	115*		
52	Chronic hypertrophic arthritides	M	42	30	64	93*		
53	Psychoneurosis	F	37	29	83	132*		
54	Pleurisy with effusion	M	24	28	54			
55	Arteriosclerosis	M	68	28	60	108*		
56	Angina pectoris cardiac decompensation	M	71	27	66	111		172*

TABLE I—Continued

Case	Diagnosis	Sex	Age	Total sugar glycolyzed Hours				
				2	4	6	8	10
57	Arsenical hepatitis	M	39	26	60			
58	Carcinoma of stomach	M	61	26	76			
59	Senile cataract	F	63	25	46	80		
60	Diabetes mellitus	F	60	24	70	94	118	165
61	Chronic myocarditis, hypertension	M	47	24	76			
62	Senile cataract	F	74	24	57	68	102*	
63	Choroiditis	F	65	24	65	84	126*	
64	Lymphocytic leukemia	F	24	23	64			
65	Pulmonary tuberculosis, tubercular peritonitis	M	31	23	54			
66	Lymphocytic leukemia	M	20	23	64	126*		
67	Polycythemia vera	M	44	22	62			
68	Syphilis	M	63	22	44	65*		
69	Myelogenous leukemia	F	66	22	79			
70	Senile cataract	M	70	21	71	95	129*	
71	Pernicious anemia	F	27	21	42			
72	Pernicious anemia	M	68	21	71			
73	Gangrene toe	M	50	20	64		117*	
74	Gangrenous appendix, pelvic abscess	M	47	20	85	126*		
75	Latent syphilis, peptic ulcer	M	32	18	63			
76	Diabetes mellitus	M	34	18	60	113	138	170
77	Adenoma of thyroid	F	32	18	85		138*	
78	Chronic appendicitis	F	25	16	43	75	107	138*
79	Cardiac decompensation	M	43	15	47	101	140*	
80	Diabetes mellitus, cardiac decompensation, chronic myocarditis	M	43	11	61	98	128*	

\* Initial blood sugar the blood was completely glycolyzed when analyzed at the end of this period

Stained smears were made from each specimen of the blood at the end of each experiment, and in no case were microorganisms found. Complete blood counts were obtained on the day of the study.

#### RESULTS AND DISCUSSION

The rates of glycolysis in 80 specimens of blood from patients with widely different diseases are recorded in Table I. It will be observed

that the amount of sugar glycolyzed during a two hour period of observation varied from 11 mgm to 98 mgm per 100 cc of blood. The variation in the glycolytic activity of different bloods is more evident in this table than in previously reported data. There are instances notably those reported by Falcon Lesses (3) and by Schmitz and Glover (4), in which a large amount of sugar was glycolyzed. Likewise there are observations in which the rate of glycolysis was very low (Birchard (5), White and Watson (6), John (7), and Lemann and Liles (8)). There is in this study no correlation of the age or sex of the patient and the rate of glycolysis.

An examination of the methods used will do much to explain the variation in results obtained by different investigators. The most important cause for the diversified results in glycolytic studies has been the use of anticoagulants. Bürger (9) has demonstrated that the addition of citrate or oxalate affects the rate of glycolysis. Data obtained in this laboratory showed further that the glycolytic power of blood is retarded in proportion to the concentration of citrate or oxalate. Fluoride arrests glycolysis, as has been demonstrated by Major (10), and by Dickens and Simer (11). Hence all data obtained with blood to which oxalate, citrate or fluoride has been added cannot be compared with results obtained without the use of these anticoagulants. Defibrination or heparinization of blood does not affect the rate of glycolysis (3) (4) (12).

Of other extrinsic factors which might influence the rate of glycolysis, temperature is of great importance. Claude Bernard (1) employed a temperature of 15° C., Lemann and Liles (8) kept their specimens of blood between 9° and 11° C., Denis and Giles (13), Mauriac (14), and Stammers (15) all used "room temperature." Thalheimer and Perry (16), Cajori and Crouter (17), Katayama (18), Negelein (19) and Mackenzie (20) used a temperature of 37-38° C. Bürger (9) demonstrated that the optimum temperature for glycolysis was 37° C. an observation which has been confirmed in this laboratory. It is to be regretted that the data on the rate of glycolysis given by different investigators often cannot be compared because standard temperature conditions were not maintained.

The influence of the initial sugar concentration on the rate of glycolysis has been the subject of much discussion. Some investigators

(5) (7) (8) (21) have taken as the rate of glycolysis the percentage of the initial sugar content which is glycolyzed in two hours. This is arbitrary and illogical since the absolute rate of glycolysis does not appear to be dependent upon the initial sugar concentration. In the author's blood specimens the initial sugar content varied from 65 mgm per 100 cc (Case 68) to 1084 mgm per 100 cc (Case 37), but the absolute rate of glycolysis in the blood of the latter patient with its excessive amount of sugar was well within the limits of normal for this series. Further, it was observed (Table II) that the rate of glycolysis

TABLE II  
*Glycolysis of excessively high blood sugar  
(Case H M)*

Hours	Blood sugar	Sugar glycolyzed
	mgm per 100 cc	mgm per 100 cc
0	800	0
2 0	725	75
4 0	655	145
6 0	585	215
10 0	558	242
18 5	455	345
23 0	312	488
41 5	120	680
65 5	45	755
72 0	0	800

continues to be about the same for six hours, even though the concentration of sugar is decreasing steadily. The decrease in the rate of glycolysis which occurs after six hours is probably due to mechanical and chemical damage to the blood cells. Cajori and Crouter (17) and Macleod (22) have demonstrated that exogenous glucose is glycolyzed at the same rate as endogenous blood sugar. This fact has been confirmed repeatedly in this laboratory. As additional proof of the lack of influence of the initial blood sugar content on the rate of glycolysis Table III is inserted to call attention to the glycolytic rate of the blood of a patient with diabetes mellitus both before and during management by diet and insulin.

The effect of insulin on the rate of glycolysis is also of interest. Three cases are presented (Table IV) to demonstrate the effect of insulin. In these cases, blood was withdrawn from the vein of one

TABLE III  
*Glycolysis before and during diabetic management  
 (Case D F B)*

Hours	Before diabetic management		During diabetic management	
	Blood sugar	Sugar glycolized	Blood sugar	Sugar glycolized
	mgm. per 100 cc.	mgm. per 100 cc.		mgm. per 100 cc.
0	372	0	277	0
2	341	31	239	38
4	300	72	198	79
6	265	107	140	137
8	197	175	118	159
10	120	252	73	204
12	102	270	34	243
14	67	305		

arm and without removing the needle insulin was injected directly into the vein. After three minutes blood was withdrawn from the vein of the other arm. Glycolysis was allowed to proceed in the usual way. It will be observed that the rates of glycolysis are parallel and that there is no indication of an increased glycolytic rate due to the direct action of insulin when it is added to the blood in this manner. These results are in accord with the work of Eadie Macleod and Noble (23) who found no evidence of a modified glycolytic rate in the blood of dogs drawn before and after the subcutaneous administration of insulin. Raab (24), Bierry, Rathery and Kourilsky (25), Ducceschi (26), Hepburn and Latchford (27), and Lemann and Liles (8) noted no change in the amount of glycolysis after insulin. Mauriac and Aubertin (28) concluded that insulin acts without changing the glycolytic power of the tissues. The authors' results do not support the work of Achard (29), who maintained that insulin acts by decreasing hyperglycemia and increasing the rate of glycolysis, nor of Thalhimer and Perry (16), who stated that the amount of glycolysis varies directly with the administration of insulin.

Under standard conditions of technique there are marked variations in the rate of glycolysis as is evident from a study of Table I. The plasma is the only blood element which is incapable of displaying any glycolytic activity if incubated under sterile conditions for several days, its original sugar content remains unchanged. Plasma is not

## STUDY OF GLYCOLYSIS

TABLE IV  
*Influence of insulin on glycolysis*

essential as a carrier of glucose for the cells the author has shown that Tyrode's solution may be substituted for it without affecting the glycolytic rate, and Kawashima (30) has substituted physiological salt solution and Locke's solution with equal success Rona and Doblin (31) and von Noorden (32) attributed some glycolytic power to blood serum but it is conceded by Irving (12) Milne and Peters (33) and Macleod (22) that neither blood serum nor plasma is able to glycolyze any of the sugar which they contain

The erythrocytes are the cause for the disappearance of much of the sugar in vitro Bloods containing large numbers of erythrocytes glycolyze sugar more rapidly than bloods containing fewer red blood corpuscles other things being equal The results of a typical observation of the rate of glycolysis in a normal blood and in the same blood when the cellular elements are diluted with its own serum are presented in Table V In Table VI the glycolytic rates of anemic bloods

TABLE V  
*Effect of diluting blood on glycolysis  
(Case H B)*

Hours	Whole blood 46 per cent corpuscles 54 per cent plasma		Modified blood 30 per cent corpuscles 70 per cent plasma		"Modified" blood 23 per cent corpuscles 77 per cent plasma	
	Blood sugar	Sugar glycolyzed	Blood sugar	Sugar glycolyzed	Blood sugar	Sugar glycolyzed
0	mgm per 100 cc 155	mgm per 100 cc 0	mgm per 100 cc 159	mgm per 100 cc 0	mgm per 100 cc 186	mgm per 100 cc 0
2	122	33	130	29	162	24
4	77	78	96	63	137	49

TABLE VI  
*Effect of concentrating anemic blood on glycolysis  
(Case B A)*

Hours	Whole blood 21 per cent corpuscles 79 per cent plasma		"Modified" blood 39 per cent corpuscles 61 per cent plasma	
	Blood sugar	Sugar glycolyzed	Blood sugar	Sugar glycolyzed
0	mgm per 100 cc 136	mgm per 100 cc 0	mgm per 100 cc 132	mgm per 100 cc 0
2	115	21	88	44
4	94	42	51	81

are compared with the rates observed in the same specimens after removal of enough serum to bring the cellular concentration approximately to normal. These experiments confirm Harrop's (34) demonstration that the erythrocytes have glycolytic power, and indicate that their concentration is important. These results are in accord with those reported by Kawashima (30) and by Doyon and Morel (35). Falcon-Lesses (3) and Cook and Somogyi (36) reached a similar conclusion after studies of the glycolytic rate in erythremia.

Not only is the concentration of erythrocytes a factor in glycolysis, but the nature of the red blood corpuscles is important. It has been demonstrated (37) that in diseases of the hematopoietic system characterized by great bone marrow activity and the releasing into the blood stream of great numbers of young and even immature red blood corpuscles there is a marked increase in the amount of sugar glycolyzed. In this connection it is interesting to note that Harrop (34), Morawitz (38) and Derra (39) have reported the increased use of oxygen by immature erythrocytes. No attempt was made in this study to correlate the rate of glycolysis and the oxygen consumption but, in view of the conclusions drawn by Glover, Daland and Schmitz (40) regarding the oxygen utilization and glycolytic rate of normal and leukemic leucocytes, such a study is desirable.

Of importance, also, in the study of glycolysis is the glycolytic power of the leukocytes. Although they exist in blood in relatively small numbers, they have a high glycolytic power, as may be demonstrated by the increased rate of disappearance of sugar accompanying slight leukocytosis. Table I shows the high glycolytic rate characteristic of leukemia. This observation has also been made by Glover, Daland and Schmitz (40), Falcon-Lesses (3), and Lepine and Boulud (41). However, as pointed out by Burger (9), Falcon-Lesses (3) and Schmitz and Glover (4) in their observations of chronic myelogenous leukemia and lymphatic leukemia, the amount of sugar glycolyzed is not necessarily in proportion to the total number of leucocytes present. This suggests that the various types of leucocytes have different glycolytic abilities, and that it is the young leucocytes (particularly the cells of the myelocytic series) which possess the greatest glycolytic power. This is revealed very clearly in Table I and has been emphasized by all investigators studying glycolysis in leukemic bloods. Lymphocytes,

on the other hand, possess a lower glycolytic activity, as is demonstrated by the fact that the blood in lymphatic leukemia utilizes much less sugar per unit of time, although the number of white blood cells may be as great as in myeloid leukemia Mauriac (42) Bürger (9), Macleod (43) and the author have shown that polymorphonuclear leucocytes are endowed with a much greater glycolytic power than mononuclear leucocytes.

It may be postulated that the endothelial cells have glycolytic ability, but these cells occur in the blood in such small numbers that they can be of very little importance in the utilization of sugar. Blood platelets which Warburg (44) states have a measurable oxygen consumption can scarcely be much of a factor in glycolysis, especially if they are effectively removed with the fibrin during defibrination as Harrop (34) believes.

#### SUMMARY AND CONCLUSIONS

1 A study of the rate of glycolysis in bloods from 80 different patients with widely different diseases is presented.

2 There is no correlation of the age or sex of the patients and the rate of glycolysis.

3 The glycolytic rate is markedly influenced by temperature. Glycolytic studies should be performed at 37° C—the optimum temperature for the disappearance of sugar from blood *in vitro*.

4 Citrates and oxalates, when used as anticoagulants, retard glycolysis in drawn blood in proportion to the amount in which they have been added.

#### Fluorides arrest glycolysis

Defibrination or heparinization does not interfere with the rate of glycolysis, and blood so treated should be used for glycolytic studies.

5 The rate of glycolysis bears no relationship to the initial glucose content of the blood.

6 Glycolysis *in vitro* is not affected by insulin.

7 Both erythrocytes and leucocytes are responsible for the glycolytic power of blood, and their number, type, age, and physiological integrity are important factors.

8 Glycolysis is a complex process involving certain variables which must be controlled if the results are to be correct.

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## THE EFFECT OF INSULIN HYPOGLYCEMIA ON THE CIRCULATION<sup>1</sup>

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Several observers have directed attention to the fact that insulin hypoglycemia may precipitate symptoms and signs of serious disturbance in the cardiovascular system. Gigon (1), in 1923 reported that a patient with diabetes mellitus had died from myocardial failure after the third dose of insulin. Reinwein (2) observed two patients with diabetes mellitus and circulatory insufficiency in whom the administration of insulin caused a decided increase in the degree of congestive failure. Joslin (3) and Blotner (4) reported cases in which cardiac infarction developed shortly after the administration of insulin, and von Noorden and Isaac (5) observed several patients with coronary artery disease in whom insulin hypoglycemia apparently was the cause of death. Several instances have been recorded in which insulin hypoglycemia precipitated typical attacks of angina pectoris (6, 7, 8).

Further evidence of altered cardiac physiology during insulin hypoglycemia is afforded by the electrocardiographic studies of Middleton and Oatway (9) and others. The changes observed consisted of diminished amplitude or inversion of the T wave.

Although the effect of insulin shock on the pulse rate and arterial blood pressure of man has been studied by several investigators (10, 11, 12, 13), but little attention has been paid to the effect of the hypoglycemic state on the minute volume output of the heart. In the only reported measurements, Lauter and Baumann (13), using the ethyl iodide method of Henderson and Haggard (14), recorded an increased circulatory minute volume during hypoglycemia in five

<sup>1</sup>This investigation was aided by a grant from the DeLamar Mobile Research Fund of Harvard University

TABLE II

*Protocol of a representative experiment*

Subject 5 Aet 16 Height 64 inches Weight 115 pounds

Time	Blood sugar mgm per 100 cc	Pulse rate per min	Arterial pressure mm Hg	Vital capacity cc	O <sub>2</sub> gen con- sumption per minute cc	Arterio- venous oxy- gen differ- ence volumes per cent	Circu- latory minute volume liters	Remarks
7.35	106			2900				
7.40		70	114/78					
7.50		72	108/72					
8.00		66	104/74					
8.07		68	104/70					
8.15		68	104/74					
8.20						6.0	3.8	Skin warm and moist No tremor No capillary pulsation
8.27				225				
8.43						6.0	3.8	
8.46								Insulin units 60 given subcutaneously
8.50		68	106/78					
9.00		70	106/74					
9.10		76	106/72					Slight tremor
9.20		72	112/78					Hunger
9.30		80	110/36					Slight dizziness and nervousness Pallor Definite capillary pulsation
9.35	67	80	108/38					Perspiring profusely Pupils dilated Weakness
9.45						5.3	4.4	
9.49					235			
9.58		80	108/38					
10.04				2800				
10.05								
10.09		71						
10.10								Orange juice 500 cc

During hypoglycemia, the minute volume output of the heart increased in every subject, although occasionally not beyond the limits of error. The increase averaged 29 per cent and varied in different individuals from 3 per cent to 86 per cent. No correlation between

the rise in circulatory minute volume and the fall in blood sugar level could be demonstrated. The pulse pressure increased in all subjects, the average change being 29 mm of mercury. Usually the systolic blood pressure rose, while the diastolic fell. Capillary pulsation was observed in 12 of 13 subjects examined for its presence. In 11 instances there was a distinct rise in pulse rate, averaging 15 beats per minute. Average values for pulse rate, arterial pressure, pulse pressure and circulatory minute volume before and during hypoglycemia are presented in Table III.

TABLE III  
Average values before and during hypoglycemia

	Pulse rate <i>per minute</i>	Arterial pressure <i>mm Hg</i>	Pulse pressure <i>mm Hg</i>	Circulatory minute volume <i>liters</i>
Before insulin	68	114/74	40	3.56
During hypoglycemia	78	128/59	69	4.58
Percentage change	+15	+12/-20	+73	+29

In five individuals the vital capacity decreased by 200 to 500 cc., while in two it increased by more than 100 cc.

#### DISCUSSION

The results of the present investigation furnish a rational explanation for the occurrence of cardiovascular disturbances during insulin hypoglycemia. They indicate that hypoglycemia usually is attended by an increase in the minute volume output of the heart, the pulse pressure and the ventricular rate. These findings denote a considerable increase in the amount of cardiac work. In normal individuals the heart easily accomplishes this increased work. In subjects with diminished myocardial reserve, however, the added work may give rise to conspicuous signs and symptoms of myocardial insufficiency. In other individuals attacks of angina pectoris may be precipitated.

In connection with the chain of events whereby insulin hypoglycemia leads to the observed changes in the circulation, certain observations on animals by Cannon, McIver and Bliss (17) are of considerable interest. According to these investigators the symptoms and signs of insulin shock result from stimulation of the sympathetic

division of the autonomic nervous system. In cats whose hearts had been denervated completely and in which one adrenal gland had been removed and the other denervated, the heart rate did not increase during hypoglycemia as it did in animals with denervated hearts but with intact adrenal glands. Cannon, McIver and Bliss, therefore, concluded that the increase in heart rate under these conditions results from a discharge of adrenine in response to stimulation of the sympathetic nerves to the adrenals.

Clinically, the changes in pulse rate, systolic and diastolic blood pressure and cardiac minute volume output that occur during insulin hypoglycemia are similar to those that follow subcutaneous administration of epinephrine (18, 19). Capillary pulsation also may appear in both conditions (12, 20). According to Boothby and Wilder (21), insulin has no direct effect on the metabolic rate. They suggested that the increased metabolism recorded during hypoglycemia results from a spontaneous discharge of epinephrine. These observations lend further support to the hypothesis that the circulatory changes observed during hypoglycemia in man result mainly from stimulation of the sympathetic system and increased discharge of adrenine.

The increased cardiac work incident to hypoglycemia probably is of particular importance if hypoglycemia develops during the treatment of diabetic coma. The observations of Foster (22) suggest that, in the presence of ketosis, the heart is less able than normally to accomplish the work demanded of it. He observed myocardial degeneration at necropsy in patients dying in diabetic coma. The weakened heart action frequently observed clinically in diabetic coma is in harmony with these findings. In the light of these observations it is not altogether surprising that patients may die from myocardial failure in spite of the fact that the manifestations of diabetic coma have been treated successfully with insulin (13).

In brief, not only is the heart called upon to perform more work in insulin hypoglycemia, but in certain subjects it is probably less capable than normally of responding to these increased demands. The results of the present investigation suggest that great care should be taken to avoid hypoglycemia in subjects with arteriosclerosis, angina pectoris, or symptoms and signs of circulatory insufficiency, particularly when ketosis is present. Under such circumstances, hypoglycemia usually

can be avoided by giving smaller amounts of insulin at more frequent intervals, by repeated measurements of the blood sugar content and, when necessary by the administration of glucose

#### SUMMARY

1 The effect of insulin hypoglycemia on the pulse rate, arterial blood pressure minute volume output of the heart and vital capacity of the lungs was measured in sixteen normal non-diabetic individuals

2 The pulse rate and pulse pressure increased during hypoglycemia the systolic blood pressure rising, while the diastolic fell

3 The minute volume output of the heart increased during hypoglycemia

4 These observations indicate that insulin hypoglycemia is attended by an increased amount of cardiac work

5 This increased cardiac work furnishes a rational explanation for the clinical observation that, in subjects with diminished myocardial reserve hypoglycemia may result in the development of conspicuous signs and symptoms of myocardial failure It also serves to explain the occurrence of attacks of angina pectoris in certain individuals during hypoglycemia

6 The results of the investigation suggest that great care should be taken to avoid hypoglycemia in subjects with arteriosclerosis, angina pectoris, or with symptoms and signs of circulatory insufficiency, particularly when ketosis is present

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# STUDIES ON THE RELATIONSHIP BETWEEN OXYGEN CONSUMPTION AND NITROGEN METABOLISM I IN PERNICIOUS ANEMIA

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In this communication we are reporting the first of a series of metabolic studies in certain diseases in which there is an altered rate of oxygen consumption without a recognized disturbance in thyroid function. We wish particularly to stress a correlation between oxygen consumption and protein metabolism. Data obtained by a study of six patients with pernicious anemia are presented and discussed.

Many metabolic studies have been made in pernicious anemia. Some of the factors which were most disturbing in the earlier work are now controllable. We refer to the fact that remissions in the disease can be brought about rapidly and consistently by therapeutic measures and such remissions can be recognized early by means of the reticulocyte crisis. Our primary concern has been to determine and to explain, if possible, the variations in oxygen consumption during relapses, reticulocyte crises, and regenerative periods in pernicious anemia.

## REVIEW OF LITERATURE

Observations on the oxygen consumption in patients with pernicious anemia are quite numerous in the literature. The earlier reports were for the most part, only single basal metabolic rates which were normal or moderately increased. Such observations were made by Meyer and DuBois (1) Tompkins Brittingham and Drinker (2) Grafe (3) and Boothby and Sandiford (4). Meyer and DuBois suggested that the oxygen consumption was increased in severe anemia. Later some investigators made repeated examinations on the same patient and learned that the oxygen consumption usually decreased as the blood count increased. Among these observers were Becker (5) Richards and Strauss (6), Alt (7), and Grassheim (8).

Previous to 1926 studies on nitrogen metabolism in pernicious anemia

gave conflicting results. Some patients would show nitrogen retention and others nitrogen loss, and occasionally a shift from negative to positive nitrogen balance would occur in a patient while under observation. It was demonstrated that the nitrogen balance was not necessarily dependent upon the hemoglobin content of the blood or the protein intake. Studies were made before and after splenectomy and considerable effort was made to determine the proportion of nitrogen eliminated as urea and uric acid, but in many instances it is not possible now to tell whether the disease was in a regenerating or a relapsing phase when these observations were made. Gibson and Howard (9) obtained nitrogen retention by giving a diet (10) containing fifty grams of liver per diem. Alt recently showed that the

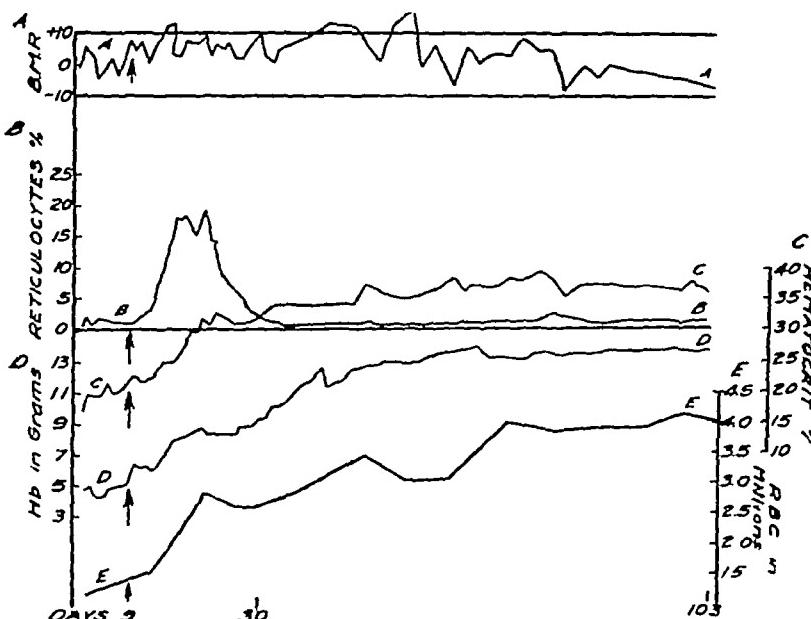


CHART 1 CASE 1

Case 1, L. M., female, aged 46 years. The total period of observation was 103 days. Determinations were made daily for 34 days, once during the sixth week and tri-weekly thereafter except that the basal metabolic rate was not done on the fifth day and no observations were made between the eighty-sixth and ninety-eighth days. Erythrocyte counts were made frequently. The treatment consisted of 4 cc of dilute hydrochloric acid with each meal and three vials of Lilly's liver extract daily, beginning after a control period of 9 days. General hospital diet was given for the first 36 days and high caloric general diet for the succeeding 67 days. The body weight increased from 53.0 kgm to 63.5 kgm. During the period 57 determinations of the basal metabolic rate were made with duplicate gas analyses.

nitrogen balance shifts from a negative to a positive phase soon after treatment with liver extract is begun. However, one might rightly object to Alt's assumption that the stools contain one gram of nitrogen per diem (See data on Case 6). No doubt the earlier and very careful metabolic studies yielded variable results because the observers lacked the present methods for producing and recognizing remissions.

The water balance in pernicious anemia has been carefully studied by Meulengracht and his associates (11) who found that water is often retained during relapses and excreted normally during remissions. It is their contention that the water retention is not due to low hemoglobin content of the blood nor renal insufficiency but may be dependent upon the lowered colloid osmotic pressure of the plasma. Alt found a decrease in urinary

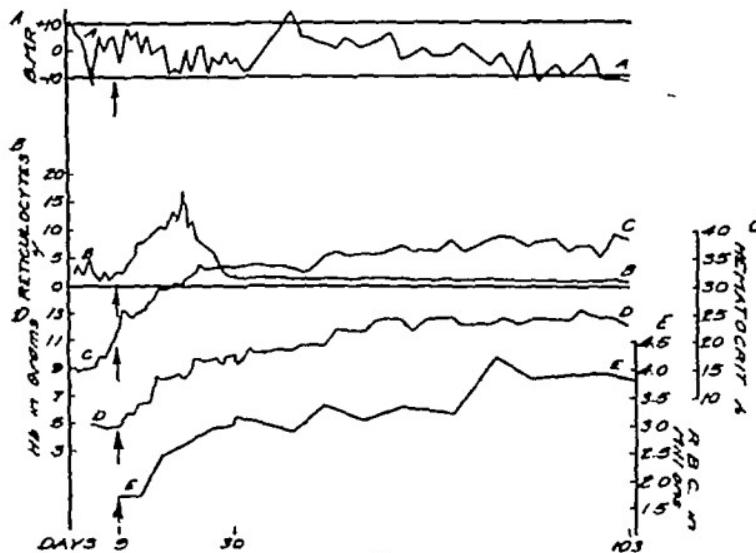


CHART 2 CASE 2

**Case 2**, L. D., female aged 50 years. The total period of observation was 103 days. Determinations were made daily for 34 days once during the sixth week and tri weekly thereafter. Frythrocyte counts were made frequently but not daily. Treatment consisted of 4 cc of dilute hydrochloric acid with each meal and of 45 cc. of Armour's liver extract daily after a control period of 9 days. General hospital diet was given for the first 36 days and a high caloric general diet for the succeeding 67 days. The body weight increased from 44.2 kgm to 56.2 kgm. During the period 61 determinations of the basal metabolic rate were made.

volume during the reticulocyte crisis with an increased output later in the regenerative period. An accurate water balance is very difficult to obtain because the factors which ordinarily affect the imperceptible water loss are hard to control over long periods. The amount of imperceptible water loss is further disturbed if there be a changing basal oxygen consumption.

#### METHODS AND RESULTS

Our first consideration was to determine as accurately as possible the daily variation in oxygen consumption before, during and after the reticulocyte crises of induced remissions in pernicious anemia.

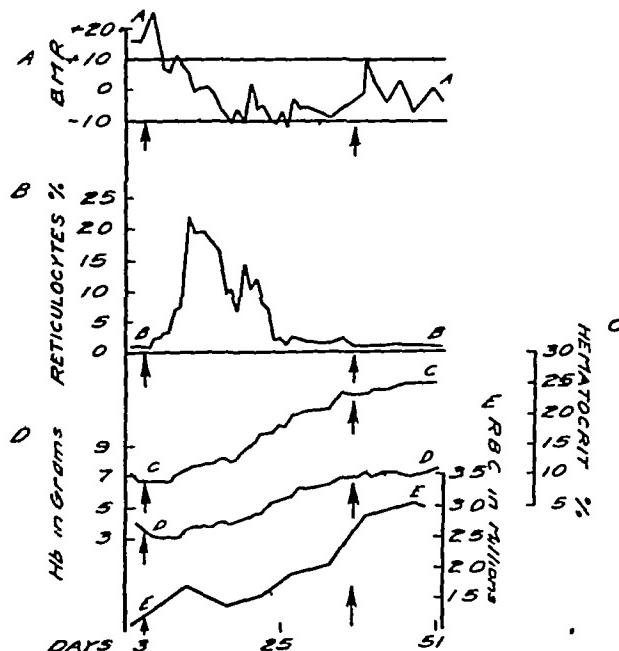


CHART 3 □ CASE 3

*Case 3, M. M., female, aged 45 years.* Determinations were made daily for the first 28 days and tri-weekly thereafter except that the basal metabolic rate was not done on the ninth day and erythrocyte counts were done periodically. The total period of observation was 50 days. Treatment consisted of 2 cc of dilute hydrochloric acid with each meal and 1.5 grain of luminal daily for the first 34 days. The patient also received 45 cc of Armour's liver extract daily after a control period of three days. On the thirty-sixth day the dose of liver extract was doubled. The diet varied considerably during the control period because of nausea and some vomiting. The body weight increased from 38.8 kgm to 43.0 kgm. A total of 37 determinations of the basal metabolic rate was made.

For this purpose six cases of typical and severe pernicious anemia were selected. In one patient the nitrogen balance was also determined because this seemed to be of great importance in explaining the variations in oxygen consumption.

The subjective and objective features upon which the diagnoses were based can be expressed most readily by means of the ten diagnostic points described by Rohner (12) (1) Previous remissions, (2) paresthesias, (3) glossitis, (4) subacute combined sclerosis, (5) color index greater than unity, (6) erythrocytes below 25 million, (7) leukopenia, (8) macrocytosis, (9) achlorhydria (10) positive Van den Bergh

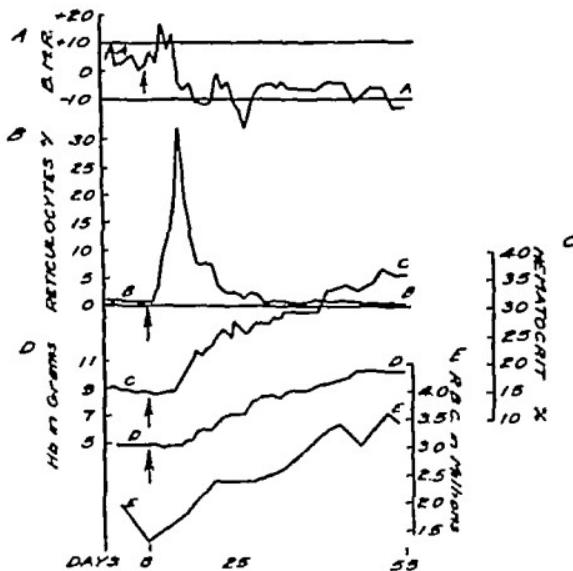


CHART 4 CASE 4

*Case 4, L. L., female aged 69 years.* Determinations were made daily for 33 days and tri weekly thereafter except that the basal metabolic rate was not done on the fifth day and erythrocytes were enumerated 12 times during the period. The total period of observation was 55 days. Treatment consisted of 2 cc. of dilute hydrochloric acid with each meal, three grains of luminal daily from the twelfth day to the end of the period and 6 vials of Lilly's liver extract daily after a control period of 8 days. General hospital diet was given. The weight increased from 50.7 kgm to 53.3 kgm. Basal metabolic rate determinations were made on 41 occasions.

In Cases 1, 2, 5, and 6 all of the diagnostic points were present. In Case 3 there had been no previous remissions and there was no evidence of subacute combined sclerosis. In Case 4 paresthesia and subacute combined sclerosis were absent.

The following determinations were made on each patient:

- 1 The basal metabolic rate by Tissot spirometer and Haldane gas analysis (average of two analyses for each reading)
- 2 Hemoglobin by the Newcomer method (19)
- 3 Hematocrit by the Van Allen method (20)
- 4 Reticulocyte counts (brilliant cresyl blue, 500 cells counted)
- 5 Erythrocyte counts

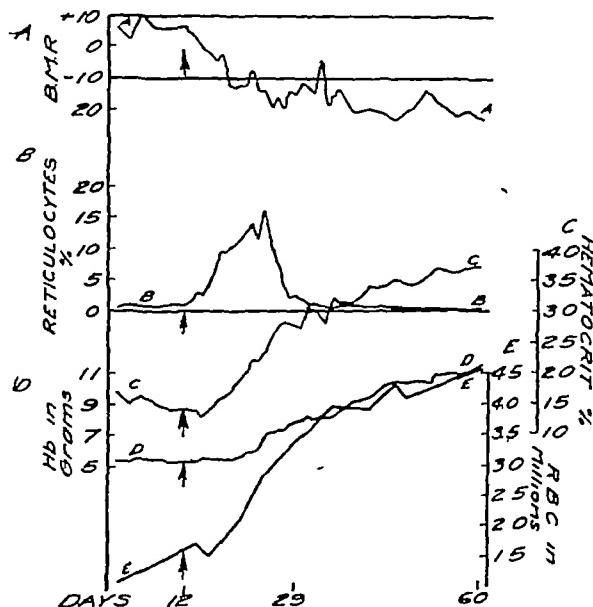


CHART 5 CASE 5

*Case 5, C S, male, aged 70 years.* Observations were made on alternate days during a control period of 12 days, then daily for 25 days and tri-weekly thereafter except that the basal metabolic rate was not done on the thirteenth day. Only 13 erythrocyte counts were made during the 60 day period of observation. Treatment consisted of 3 cc of dilute hydrochloric acid with each meal and 6 vials of Lilly's liver extract daily after a control period of 12 days. General hospital diet was given. The body weight increased from 61.6 kgm to 63.3 kgm. Forty basal metabolic rate determinations were made.

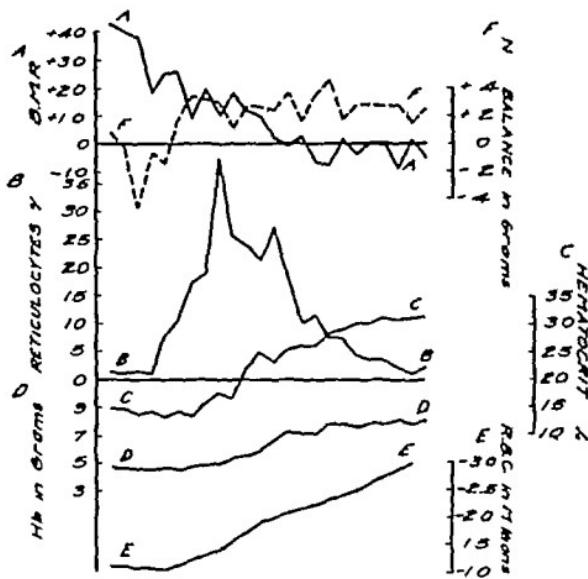


CHART 6 CASE 6

*Case 6* J. L., female aged 46 years. All observations except erythrocyte counts were made daily for 24 days which was the total period of study. In addition to the above mentioned determinations, water balance, nitrogen balance and nitrogen partition were done. Treatment consisted of 4 cc of dilute hydrochloric acid with each meal and 6 vials of Lilly's liver extract daily beginning after a control period of three days. The diet was weighed and refused portions were weighed after each meal. The body weight increased from 59 kgm to 60.3 kgm.

#### DISCUSSION

Observation of the charts on the cases in this series will show that in all save one (Case 1) there was a definite and gradual decrease in the basal metabolic rate very soon after liver extract was begun. This decrease in oxygen consumption was most marked and uniform in the last three patients in whom the dose of liver extract was double that used in the first three cases. We will first consider the ordinary factors which may disturb the basal metabolic rate.

The body temperature is usually elevated during a severe relapse in pernicious anemia and is practically normal during a remission. As can be seen from the tabulations (Table 1) the fall in temperature

TABLE 1

*Temperature, pulse, respirations, respiratory quotient, and caloric requirements of the patients studied expressed in averages for the various periods*

	Days	Temperature ° F	Standard error of the mean	Pulse per minute	Standard error of the mean	Respirations per minute	Respiratory quotient	Standard error of the mean	Calories per square meter per hour	Standard error of the mean
<b>Case I—L M</b>										
Control period	9	98.8	11	84.5	1.2	19.9	723	0.06	36.3	4
Reticulocyte crisis	20	98.5	0.8	83.1	0.8	20.0	730	0.04	38.1	2
Regeneration period	74	98.2	0.4	85.1	0.7	20.0	740	0.05	37.2	1
<b>Case II—L D</b>										
Control period	9	98.5	1.8	84.4	1.2	19.8	785	0.10	36.0	5
Reticulocyte crisis	19	98.1	0.8	78.1	1.0	19.5	812	0.06	34.4	3
Regeneration period	75	97.7	0.5	81.5	0.5	19.6	810	0.05	34.1	3
<b>Case III—M M</b>										
Control period	3	100.0	1.9	96.4	3.0	21.1	732	0.05	41.9	3
Reticulocyte crisis	20	99.4	1.0	92.2	1.2	20.4	782	0.07	35.7	5
Regeneration period	29	98.7	0.4	80.6	0.7	19.6	843	0.08	34.7	4
<b>Case IV—L L</b>										
Control period	8	99.7	1.1	86.8	2.3	20.6	773	0.09	34.4	3
Reticulocyte crisis	16	99.4	1.0	86.0	1.5	20.0	758	0.08	32.2	5
Regeneration period	31	99.0	0.6	72.5	0.8	19.3	758	0.07	30.0	3
<b>Case V—C S</b>										
Control period	12	98.5	0.9	67.4	0.9	18.5	784	0.06	37.7	3
Reticulocyte crisis	17	97.9	1.0	61.1	0.7	18.0	826	0.09	31.8	4
Regeneration period	31	97.8	0.7	64.0	0.7	19.0	846	0.12	29.1	4
<b>Case VI—J L</b>										
Control period	3	100.8	1.5	100.0	2.4	20.5	809	0.08	50.9	4
Reticulocyte crisis	16	99.2	1.1	85.7	1.3	20.3	790	0.08	39.1	7
Regeneration period	5	98.9	1.4	75.1	0.8	20.0	766	0.05	35.0	5

Figures for temperature, pulse rate, and respiratory rate represent the average of three determinations per diem for the number of days indicated. Determinations were made at 8 A M, 4 P M, and 8 P M. The respiratory quotient and calories per square meter per hour represent the average of two gas analyses for each determination during the period.

$$\text{Standard error of the mean} = \sqrt{\frac{\sum(d^2)}{n(n - 1)}}$$

was rather insignificant in most instances and certainly did not parallel the decrease in oxygen consumption. In addition, the temperature was usually most elevated in the afternoon, so that, although a morbid process which produced fever may have been present, the actual thermic disturbance was very slight or absent at the time of the test. While we can not exclude fever as a factor in increasing the basal metabolic rate at the beginning of the test periods, we can say that change in temperature was a factor of very minor or no importance in bringing about the decrease in oxygen consumption in which we are interested.

The pulse rate decreased slightly during the reticulocyte crises in some patients, but most of the decrease occurred later in the period of regeneration. The work of the heart in these patients can not be determined from the pulse rate alone because the slight slowing might be easily overbalanced by an increase in blood viscosity or in peripheral resistance. We can find no circumstance which indicates that there was any significant change in the energy utilized in maintaining blood flow during the periods of decrease in oxygen consumption in our patients.

The respiratory rate changed so little as to be of no significance.

Psychic reactions may be a factor in oxygen consumption and are very hard to evaluate. Most of our patients showed the irritability so often seen in pernicious anemia but none had definite psychoses and all cooperated very satisfactorily. Patients 3 and 4 received luminal in moderate doses without any change in the type of response. Longer control periods would have been desirable in two patients (especially Case 6) because excitement occasioned by the test is likely to increase the oxygen consumption at first. This factor, however, is not as important in pernicious anemia as in Graves' disease. In four of the patients the control periods were sufficiently long (8 to 12 days).

From the foregoing considerations we must conclude that the orderly and gradual decrease in oxygen consumption which often occurs at the beginning of an induced remission in pernicious anemia is not due to changes in the body temperature, pulse rate, respiratory rate or psychic state. The change in oxygen consumption in our cases is the reverse of what would be expected if the generally accepted explanation for the increase in oxygen consumption in pernicious anemia be correct.

Rapid cell growth and an excessive oxygen utilization by young cells have been almost universally accepted as factors contributing to the increased oxygen requirement in pernicious anemia, erythremia, leukemia and lymphoma. Obviously new cells are being formed rapidly in the last three diseases but there is some difference of opinion about the rapidity of cell formation during induced remissions in pernicious anemia.

It is to be recalled that Alt (7) reported observations which he interpreted as showing an increase in oxygen consumption during reticulocyte crises in pernicious anemia. This increase was attributed to overactivity of the blood forming tissues. Alt also found a lowered oxygen metabolism during the regenerative period when the blood forming organs must still have been rather active.

The oxygen consumption of adult erythrocytes is practically nihil, but Harrop (13) found that blood containing numerous reticulocytes consumed a measurable amount of oxygen. Accordingly the circulating blood might use more oxygen during a reticulocyte crisis than at other times, but this effect, if present, is usually completely overshadowed by some more potent influence.

That rapid cell growth in the adult organism will result in an increase in the total oxygen requirement is an assumption which seems to have gone unchallenged, but we should remind ourselves that it has not been proved, and our observations as well as those of some other investigators (14) indicate that it may be erroneous.

Rapid cell formation and rapid cell destruction coexist very commonly in diseases of the blood forming organs, and it has been very difficult to determine which of these processes is responsible for the changes in oxygen consumption which exist. Isaacs (15) suggested a relationship between the increased destruction of nuclear material and the increased oxygen consumption in erythremia, leukemia and pernicious anemia. In the first two at least and probably in all of these diseases there is rapid cell formation as well as rapid cell destruction. In a patient not reported in this series we found basal metabolic rates of 22.4 per cent and 23.2 per cent above normal during a reticulocyte crisis. At the time of these determinations 56 per cent of the erythrocytes were reticulated, and nucleated blood cells numbered 53,800 per cmm, of which 26,900 per cmm (50 per cent) were nucleated.

erythrocytes and 19,100 were neutrophiles. Unfortunately we know nothing of the oxygen consumption either just before or just after this crisis, nor do we know anything about the uric acid metabolism or nitrogen balance. Among the patients whom we studied systematically one (Case 4) showed a slight rise in the basal metabolic rate during the beginning of the reticulocyte crisis but the rate fell rapidly and was falling during the peak of the crisis. Even if all patients showed an increase in the oxygen consumption during the reticulocyte crisis we would have no proof that such an increase was the result of cell formation rather than nuclear destruction. Riddle (16) showed that the endogenous uric acid metabolism is increased in beginning remissions even before the reticulocyte crisis appears, and that the uric acid content of both the blood and urine falls before the reticulocyte crisis disappears. During the regenerative period which follows the first part of the reticulocyte crisis, we have, in pernicious anemia, a blood dyscrasia in which there is at least normally rapid cell formation without a correspondingly rapid cell destruction unless extruded nuclei are being destroyed.

Rubner (17) and Hoobler (18) separately showed that amino acids which enter into the formation of body proteins exert no specific dynamic action. When protein is stored there is also a saving of the oxygen which would have been required to metabolize it. It would seem that, during a relapse, patients with pernicious anemia are in partial protein starvation in spite of an adequate protein intake, and that by specific therapy they are suddenly rendered able to use and store nitrogen in a normal fashion. All investigators seem to agree that treatment with liver extract results in nitrogen retention regardless of any change in the protein intake. Under such circumstances the conservation of useable amino acids would seem to involve less waste (specific dynamic action) and probably less use of oxygen than would the catabolism of those amino acids.

The changes in nitrogen balance are in some instances, not great enough to account for the changes in oxygen consumption. That is, the oxygen involved in the estimated specific dynamic action together with oxygen which would have been required to metabolize the stored protein will not always be equivalent to the lowering which occurs in the total oxygen requirement. In this connection, we should re-

member the tendency of the body to retain some portions of broken down cells which can be used again (e.g., iron). It may be that the body is using certain protein fractions over and over again in an effort to perfect some type of protoplasm which is essential to normal hematopoiesis (stroma of erythrocytes?) The human organism can utilize protein derived from the body of any other animal and we are not aware of any proof that it can not re-utilize its own broken down protein. If re-utilization of protein takes place an added quantity of oxygen might be involved in the process.

Almost the converse of the above state of affairs can be demonstrated in some patients with leukemia who are given roentgen ray treatment. Results of such observations will be reported in a subsequent paper.

Several observations, some of which have been described by other investigators and which are more or less incidental to this study, will be enumerated.

1 As the blood count increased there was a tendency toward a decrease in both the volume index and the color index.

2 Serum bilirubin fell to a normal level promptly after liver therapy was begun.

3 The appetite increased soon after the reticulocyte crisis.

4 In four patients the respiratory quotient increased significantly under treatment whereas in the other two there was a decrease.

5 The urine volume averaged slightly less than the liquid intake during the control period (Table 2). During the first 6 days of liver extract therapy the urine volume decreased still more but during the remainder of the period there was a distinct diuresis. No attempt was made to measure the imperceptible water loss.

6 The uric acid excretion (Table 2) increased at the beginning of the reticulocyte crisis and continued high for several days.

7 The urea excretion (Table 2) decreased during the reticulocyte crisis but returned to its previous level early in the period of regeneration.

8 In Case 6 the nitrogen content of the stool was ten times as much for the first 6-day period as for the last period of 6 days. Much of this variation was no doubt due to diarrhea.

TABLE 2

## Nitrogen balance and nitrogen partition in Case 6

Days	Hemo-globin grams per 100 cc	Hema-tocrit per cent	Caloric intake calories	Nitrogen intake grams	Creatinine Urea	Uric acid	Ammonia	grams of nitrogen	grams of nitrogen	Nitro- gen in stools grams	Basal metabolic rate per cent	Respira- tory quotient	Liquid intake cc.	Total water intake cc.	Urine vol ume in stools, grams	Water output in stools, grams	
1	4.62	14.5	1.4	2524	10.46	1.435	5.441	.543	7.770	+0.918	+44.3	794	1500	3014	1500		
2	4.57	14.5	1.0	2541	10.56	4.381	21.70	6.312	6.83	9.014	-0.226	+41.1	799	1500	2980	1580	
3*	4.57	13.5	1.2	2376	8.70	3.875	21.88	5.233	60.4	-	-3.354	+38.7	836	1425	2846	1200	
4	4.45	14.0	9	2298	8.65	37.54	16.67	4.944	3.40	7.665	10.631	-0.592	+18.4	742	1500	2911	1000
5	4.62	13.0	7.8	2312	9.08	46.68	.3038	6.652	673	8.736	-	-1.428	+25.5	756	1500	2907	1300
6	4.43	14.0	10.8	2555	10.32	3.691	25.84	4.270	698	6.683	+1.864	+26.9	803	1450	2865	1240	
7	4.62	13.0	17.4	2572	10.32	3017	14.76	3.604	662	5.355	+3.529	+9.5	784	1500	2952	1020	
8	4.74	15.5	18.8	2572	10.32	3140	.3236	4.165	654	5.653	+3.231	+20.0	877	1500	2952	1700	
9	4.82	17.5	39.8	2572	10.32	3032	21.55	3.712	571	5.936	+2.948	+10.0	878	1800	3252	1600	
10	5.33	16.5	25.7	2559	10.32	3986	30.20	5.224	612	7.782	8.615	+1.102	+18.3	859	1800	3252	3270
11	5.50	22.0	24.0	2572	10.32	3442	17.79	4.292	505	6.224	+2.660	+12.3	761	1800	3252	2470	
12	5.75	25.0	21.6	2572	10.32	2443	12.69	4.401	539	6.164	+2.720	+10.2	812	1800	3442	2590	
13	6.60	23.0	27.0	2372	10.32	4183	24.12	5.512	608	6.894	+2.514	+2.2	793	1800	3312	2010	
14	7.11	25.0	19.0	2572	10.32	3189	1.593	4.007	500	5.670	+3.738	-0.3	755	1800	3352	2000	
15	7.02	26.0	10.0	2572	10.32	4506	23.42	5.713	673	7.787	+1.620	+2.5	774	1800	3352	2500	
16	7.03	26.0	11.6	2603	10.57	3407	11.98	4.400	355	6.080	+3.578	-6.3	745	1800	3445	1720	
17	7.70	28.0	7.7	2572	10.32	2630	14.63	3.664	452	4.775	+4.633	-6.9	760	1800	3452	2100	
18	7.70	29.0	7.4	2572	10.32	4349	19.16	5.241	670	7.570	+1.837	+1.9	769	1800	3454	2060	
19	7.44	30.0	4.2	2572	10.32	4267	1.381	5.159	642	7.355	+2.793	-3.8	784	1800	3452	2960	
20	7.78	30.0	3.4	2572	10.32	3821	14.27	4.527	482	7.271	+2.857	+0.2	746	1800	3452	2120	
21	7.70	31.0	3.4	2572	10.32	3804	16.56	5.802	488	7.373	+2.735	-0.1	769	1800	3452	2290	
22	8.04	30.5	1.8	2572	10.32	4037	14.71	5.166	.556	7.291	+2.836	-8.6	759	1800	3452	2240	
23	7.70	31.0	1.0	2572	10.32	4188	17.40	7.030	499	8.647	+1.480	+1.4	790	1800	3452	2130	
24	7.70	31.0	2.0	2572	10.32	3787	17.25	5.955	617	7.373	+2.755	-5.5	766	1800	3452	2290	

\* On the third day of observation there was an emesis containing 597.7 grams of water and 2.7222 grams of nitrogen. Note.—Stools were collected for a period of six days and daily nitrogen balances were obtained by adding one sixth of the stool nitrogen for the period to the urinary nitrogen for each day.

## SUMMARY

A blood dyscrasia (pernicious anemia) has been studied during relapses and remissions. Early in induced remissions the influences which cause a lowering of the oxygen requirement are usually potent enough to counteract the effect of two factors which might in themselves tend to increase the oxygen requirement during this period. These two factors are, first, the increased oxygen requirement of circulating reticulocytes, and, second, the increased oxygen required to maintain the high level of endogenous uric acid metabolism which exists. During an active relapse blood cells are being destroyed more rapidly than they are formed, and there is a negative nitrogen balance with an increased oxygen consumption. During induced remissions blood cells are being formed much more rapidly than they are being destroyed and there is nitrogen retention with a decreasing oxygen consumption. We wish to suggest the following as a working hypothesis. There is a direct causal relationship between the increase in nitrogen catabolism and the increased oxygen consumption, and a similar relationship of cause and effect between nitrogen storage and decrease in oxygen requirement.

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## BREATHING MEASUREMENTS ON NORMAL NEWBORN INFANTS

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### INTRODUCTION

Observations upon the frequency and amplitude of the respiratory movements of newborn children, recorded in the literature, are conflicting. The rate of breathing per minute is recorded as follows: Dohrn (1), 62; Eckstein and Rominger (2), 37 to 49; Pfaundler and Schlossmann (3), 40 to 45, and Feldman (4), 44.

The depth of breathing expressed in cubic centimeters, is given by Feldman (4) as 48; Dohrn (1), 45; Pfaundler and Schlossmann (3), 27 to 42; von Recklinghausen (5), 19.5; Gregor (6), 15, and Eckstein and Rominger (2), 10 to 13.

The amount of air breathed per minute is 1300 cc according to Eckerlein (7), while Eckstein and Rominger (2) record 600 to 1000 cc. The minute volume per kilogram of body weight is 400 cc. as reported by Feldman (4) while Pfaundler and Schlossmann (3) say that it varies from 330 to 500 cc.

Observations have been made recently with the apparatus shown in Figure 1 which are the first to be recorded by this method.

### APPARATUS

The features of the apparatus which made possible the accuracy of the method were the size and construction of the Krogh spirometer and the collar arranged to render the system air-tight and the apparatus rigid. The float of the Krogh spirometer is constructed of sheet aluminum 1/5000 of an inch in thickness. Its top measures 9.5 by 7.8 cm. The writing point end of the float extends into the water a dis-

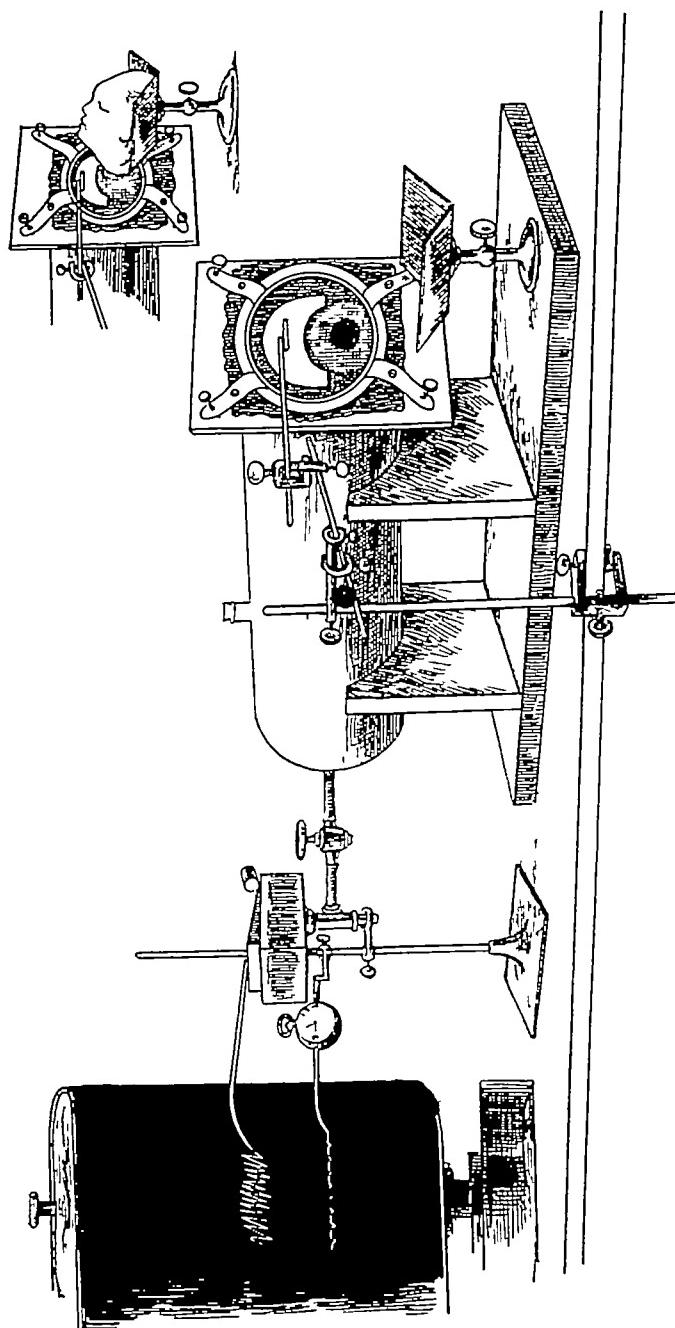


FIG 1 APPARATUS FOR MEASUREMENT OF RATE AND DEPTH OF RESPIRATORY MOVEMENTS  
OF NEWBORN INFANT

Note the collar arrangement for creating an air-tight seal about the neck. The central portion of the collar is thinner than the peripheral part. The fenestration being smaller than the neck, the rubber must be stretched. This increases its rigidity, which is further augmented by pressure from the metallic crescentic-shaped shutter.

tance of 5.3 cm, the opposite end 2 cm. The rubber collar<sup>1</sup> is 25 cm in diameter. The peripheral portion is 2.5 mm thick, the central portion 5 mm. The central opening is 2.5 cm in diameter.

Calibration of the spirometer using a burette and a gravity bottle for water displacement, indicated that a movement of 10 cc of air would alter the pointer of the spirometer a distance of 7 mm. No difference in the calibration was noted when the plethysmograph was included in the system with appropriate fixation of the rubber collar, to simulate that effected when the infant was in position.

To determine the error introduced by *rapid* changes of air volume, further calibration was carried out by the use of a new Record syringe. The latter by calibration was set to transfer 21.4 cc of air per stroke. Using very slow movement of the piston the position of the spirometer pointer moved a distance of 13.0 mm for each 21.4 cc of air transferred. Employing an alternating filling and emptying of the spirometer at a rate of 54 fillings per minute 15.2 mm displacement of the pointer was the average produced per stroke whereas a rate of 104 per minute increased this movement to 15.5 mm per average stroke.

With the plethysmograph in the system, the neck opening of the collar plugged with a beaker, the crescentic shutter in place and the lower part of the collar also made rigid by slight constant pressure against the beaker calibration was repeated. Moving the syringe at a rate of 54 strokes per minute the pointer moved 15 mm on the average, while a rate of 104 gave a pointer excursion of 15.1 mm.

The observations were carried out upon infants born in the University of Pennsylvania Hospital during the months of June, July, August and September 1930. Tests were made within the first day or two of birth when possible (Table 3). The selection of infants was based upon size and the fact that careful physical examination revealed no abnormalities. Of 74 tested infants one later exhibited signs of parathyroid tetany and three developed a mild degree of dehydration fever. Three quarters of the entire group of 74 infants were observed by one of us in the outpatient department for the three months following the breathing tests and were normal throughout that time. The

<sup>1</sup> This portion of the apparatus is standard equipment for the infant size Drinker respirator and can be purchased through the Warren E. Collins Co. of Boston, Massachusetts.

breathing of the infant having tetany did not deviate from the average in any respect

#### PROCEDURE

To secure the quietest breathing and therefore a record of minimum ventilation, an attempt was made to measure only sleeping infants. Tests were done immediately after feeding time. If the infant was not sleepy it was given an additional feeding of 5 per cent Karo syrup in warm water. Weight and body measurements were recorded (Tables 1 and 4). A continuous tracing was made attempting to secure a record of from 10 to 20 minutes of breathing during sleep. Sleep was not always continuous, however, throughout the period. Each infant was measured only once.

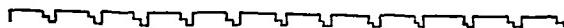
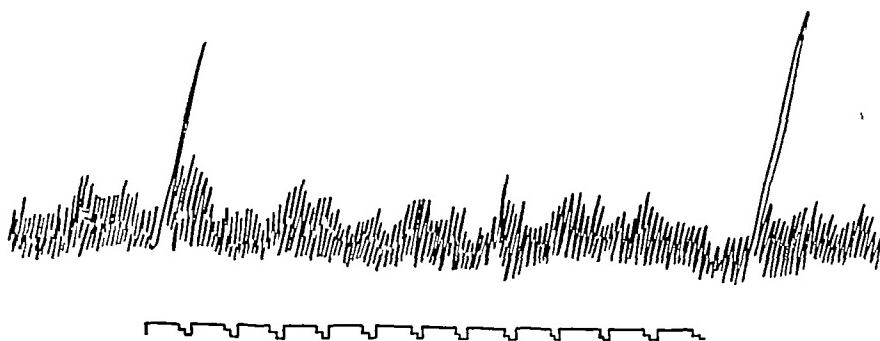
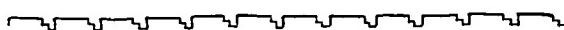
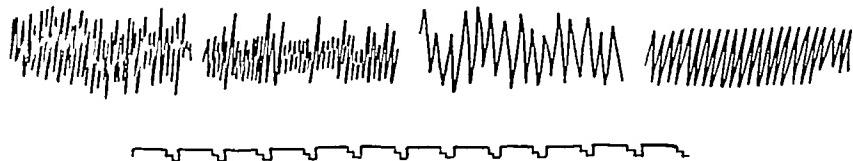


FIG 2 PLETHYSMOGRAPHIC RECORDS MADE ON 5 SLEEPING INFANTS UNDER 48 HOURS OF AGE SHOWING THE CHARACTERISTIC IRREGULARITIES OF THE BREATHING RHYTHM

Note the two deep sighs in the lower tracing. Time marker records 5 second intervals

After the tracings were shellaced and ready for examination selected parts showing the slowest and most uniform breathing from the record of each infant were chosen in the following manner. Those parts of each tracing made during sleep were subdivided into samples one-half

minute in length. Avoiding the choice of consecutive samples, the three samples showing the slowest breathing rates were selected and each was measured in the following manner. Using a specially calibrated rule, each inspiratory stroke of the sample was measured with dividers, and the summation for the half minute doubled to give the

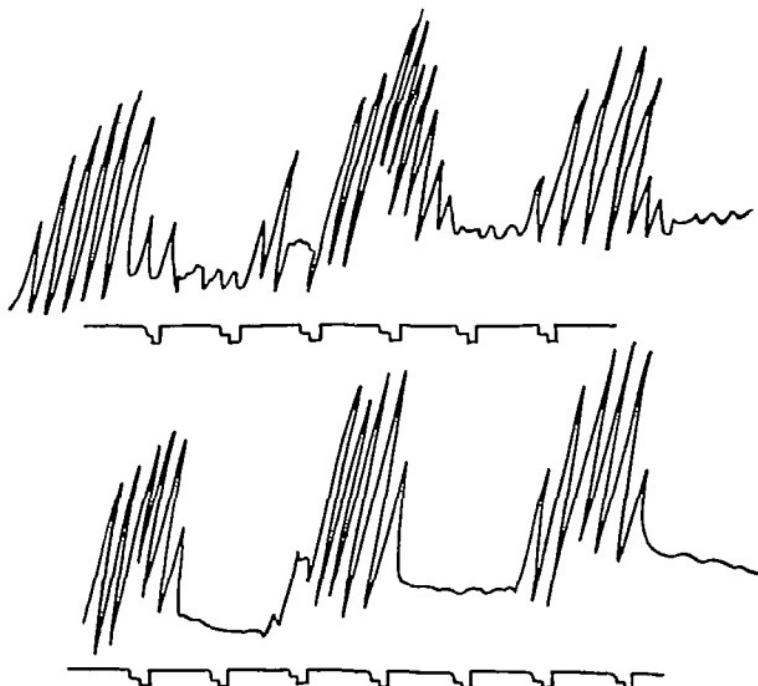


FIG. 3 SHOWING CHEYNE STOKES TYPE OF BREATHING ALSO IN AN INFANT UNDER 48 HOURS OF AGE

This is rarely seen usually just at beginning of sleep and is very transient. Time marker records 5 second intervals.

minute volume. The three minute volume figures secured in this manner were compared. If the largest was less than 10 per cent greater than the smallest it was assumed that a suitable degree of breathing stability had been reached for the purpose of the study, and the record of this infant was placed in Table 1. Records which failed to meet this requirement are given in Table 2. Two examples of characteristic tracings are shown in Figures 2 and 3.

TABLE I

Data upon 50 healthy full-term infants whose respiratory movements were studied in the apparatus shown in figure 1. Forty seven infants were delivered, and 3 through a wide-gauge comparable records. The body measurements were made the day of the test.

Serial num- ber	Dally days*	Age	Weight	Full length	Sitting height	Chest circum- ference at nipple line	Karo syrup given	Breathing sample			Average			Difference between first and second minute volume	per cent				
								A		B	C	Minute respiratory rate	Minute vol- ume	Respira- tory rate	Minute vol- ume				
								hrs	min	per minute	cc	per minute	cc	per minute	cc				
1	A 1 Γ	2	1	3455	53.5	33.5	M	-	88	1249	96	1335	92	1222	92	1268	13	9.2	
2	O Γ	2	8	3740	50.5	32.5	M	+	60	880	62	920	58	902	60	902	15	1.6	
3	S	1	2	3957	50.0	33.0	M	+	116	1413	98	1354	94	1358	102	1375	13	1.0	
4	O I	20	20	2972	47.0	31.8	30.5	Γ	-	44	898	48	958	44	880	45	912	20	8.8
5	S	17	17	2508	47.0	31.0	28.5	M	-	38	566	38	557	38	574	38	565	14	3.0
6	A 1 Γ	1	5	3778	50.0	34.5	M	-	52	870	58	898	56	814	55	860	15	10.0	
7	O Γ	19	19	3429	50.0	32.0	M	-	36	617	38	634	38	606	37	619	16	4.6	
8	A 1 Γ	9	7	3451	50.4	33.0	32.5	F	-	30	730	28	730	30	781	29	746	25	6.9
9	O F	9	0	3010	47.0	30.5	30.0	F	+	46	871	48	895	48	876	47	880	18	2.7
10	O F	5	4	2695	50.5	32.0	30.5	F	+	32	613	30	592	30	572	30	592	19	7.1
11	O Γ	5	2320	50.5	32.5	30.5	F	+	30	594	30	604	30	584	30	594	19	3.0	
12	S	14	3067	47.5	31.5	32.0	M	+	48	897	48	869	48	881	48	882	18	3.0	
13	O Γ	2	1	2600	47.0	30.0	29.0	F	+	38	454	38	474	38	479	38	469	12	5.0
14	B	9	3807	51.0	33.2	33.5	M	-	42	836	42	907	44	842	42	861	20	8.0	
15	O F	4	3	2972	49.0	32.8	33.5	F	-	40	635	36	662	38	672	38	656	17	5.0
16	S	5	3989	53.5	35.0	34.5	M	-	38	830	38	887	38	829	38	848	22	7.0	
17	O Γ	16	3473	49.0	33.2	32.0	F	+	48	967	46	923	46	1001	46	963	20	8.0	
18	O F	18	2816	49.0	32.0	26.5	F	+	42	581	46	591	44	564	44	579	13	4.0	
19	O F	3	8	3590	50.0	34.0	34.0	F	+	82	986	72	929	78	993	77	969	12	6.0
20	S	15	2946	46.5	31.0	30.5	F	-	28	464	26	457	26	434	26	451	17	6.0	
21	O F	5	2760	47.0	31.5	29.5	F	-	36	606	36	609	36	579	36	598	16	5.0	
22	B	9	3650	50.0	34.0	34.0	M	+	64	840	64	838	62	817	63	831	13	2.0	
23	O F	4	16	3000	49.0	32.5	32.0	M	-	28	560	28	554	28	574	28	562	20	5.0

\* Symbols A T F Axis traction forces O F Outlet forces F "Forceps"

B Breech delivery S Spontaneous delivery + Yes - No

TABLE I—Continued

Serial number	Delivery	Age	Weight	Full length	Sitting height	Chest circumference at nipple line	Karo syrup given	Breathing samples				Average				Difference between largest and smallest minute volume per cent			
								A		B		C		D					
								Respiratory rate per minute	Minute vol. volume cc.										
24	O F	10	17	2694	47.0	30.0	31.5	F	+ + + + +	657	50	655	50	664	13	3.0			
25	O F	7	10	3378	51.0	32.5	33.5	F	+ + + + +	435	36	408	36	426	11	6.0			
26	O F	5	2921	47.5	30.8	31.5	N	- - - - -	650	34	626	30	589	32	621	19	9.0		
27	S	8	3570	50.5	34.0	34.0	F	- - - - -	42	756	42	748	42	787	42	763	18	5.0	
28	O F	5	2882	48.0	32.0	33.0	F	+ + + + +	54	599	52	632	66	629	57	620	10	5.0	
29	O F	1	21	3155	48.0	31.2	32.5	M	- - - - -	38	588	38	574	38	544	38	568	14	8.0
30	S	1	3	3370	50.5	35.0	33.0	M	- + + + +	46	678	46	698	46	637	46	671	14	9.0
31	B	1	15	3690	51.0	34.2	34.0	F	+ + + + +	60	649	58	692	62	636	58	659	11	8.0
32	A, T F	5	3112	53.5	35.2	34.5	M	- - - - -	66	973	66	943	62	916	64	944	14	6.0	
33	O F	15	3112	49.0	31.2	32.5	F	- + + + +	44	577	44	575	46	561	44	571	12	2.0	
34	S	7	3570	50.0	32.5	32.0	F	- + + + +	34	590	34	584	34	633	34	602	17	8.0	
35	S	9	3575	50.0	33.0	34.0	F	- - + + +	42	660	38	636	34	599	38	631	16	10.0	
36	S	2	17	2647	49.0	30.8	30.5	F	- - + + +	24	489	24	481	26	506	24	492	20	5.0
37	S	1	22	3131	50.0	32.5	32.0	M	- + + + +	38	670	40	669	42	699	40	679	12	4.0
38	F	2	22	3102	51.0	33.6	33.5	F	- + + + +	28	492	28	516	26	503	27	503	18	4.0
39	O F	1	3	2441	47.5	31.0	27.5	F	- - + + +	34	649	32	624	34	603	33	625	18	7.0
40	O F	1	8	3100	51.0	33.5	31.0	M	- + + + +	60	947	60	938	60	947	60	944	15	0.0+
41	A, T F	1	22	3070	50.0	31.5	31.5	F	- + + + +	32	660	32	643	32	628	19	628	19	0.2
42	S	9	3005	48.0	31.5	32.0	N	- + + + +	28	728	26	741	26	711	26	726	27	0.4	
43	S	11	3460	49.0	32.5	33.5	M	- + + + +	30	753	32	758	32	722	31	744	24	4.0	
44	O F	2	2	3235	51.5	33.0	32.0	M	- + + + +	44	746	44	760	40	712	42	739	17	6.0
45	S	4	3485	49.5	33.0	33.0	F	- + + + +	40	596	42	647	40	627	40	623	15	8.0	
46	O F	9	2970	47.5	31.0	31.0	M	- + + + +	26	496	26	513	26	534	26	514	19	7.0	
47	B	1	0	3057	50.5	32.5	32.0	F	- + + + +	34	717	34	659	34	708	34	694	20	8.0
48	S	22	3445	51.5	33.0	31.5	F	- + + + +	28	723	28	676	26	702	27	700	25	6.0	
49	F	1	0	3922	51.0	33.0	34.5	M	- + + + +	56	828	56	832	58	874	56	844	15	5.0
50	S	1	1	3605	51.0	33.5	33.0	F	- + + + +	52	861	52	858	52	858	52	858	16	0.0+

Observations upon 21 healthy full-term infants which deal with plethysmographic measurements of the frequency and amplitude of the respiratory movements  
These infants were not as yet when tested and their stability of breathing was less than that of the infants recorded in table I

TABLE 2

Serial number	Delivery	Age*	Weight	Full length	Sitting height	Chest circumference at nippit line	Karo Syrup given	Sleeping	Breathing samples				Average	Difference between first and similar minute volume	per cent	
									A		B	C				
									Respiratory rate	Minute vol. time	Respiratory rate	Minute vol. time				
51	O F	5	3310	52.0	34.0	32.5		-	52	627	50	59	per minute	cc	cc	
52	B	1	3154	50.5	33.0	32.0	M	-	56	995	60	668	70	1135	62	
53	S	1	3503	50.0	32.8	32.9	M	-	68	1017	52	774	56	862	58	
54	S	17	6920	49.0	32.0	32.0	M	+	74	917	76	986	70	824	73	
55	O F	8	3580	51.0	31.0	34.5	F	-	56	1111	42	776	40	708	46	
56	A T F	5	3516	49.0	30.6	30.2	M	+	72	805	70	778	90	1122	77	
57	O F	1	9	2764	49.0	32.3	31.1	F	-	46	820	56	1093	46	940	49
58	O F	12	4631	54.0	36.0	37.5	F	-	94	1503	96	1564	74	1215	88	
59	A T F	23	10	3304	52.0	34.0	32.2	M	-	70	1327	58	1188	68	1232	65
60	O F	10	12	3246	52.0	35.0	33.0	M	-	54	842	48	825	40	716	47
61	S	6	18	2760	48.0	31.0	31.0	M	+	36	696	38	666	38	620	37
62	O F	16	3400	49.0	33.0	32.5	M	+	44	709	42	674	52	782	46	
63	A T F	7	3315	49.5	31.8	32.5	F	-	30	720	38	799	28	602	32	
64	O F	2	9	3129	48.5	33.5	32.5	M	+	68	764	62	769	48	674	59
65	S	1	4	2629	47.0	30.5	30.0	F	+	42	945	40	661	44	810	42
66	B	1	9	3286	49.0	31.0	30.5	F	-	72	936	76	1056	58	886	68
67	F	22	3205	47.5	31.0	31.7	F	-	60	832	62	881	56	780	59	
68	O F	2	19	3444	51.0	32.5	33.5	M	-	74	1002	76	1012	62	884	70
69	S	1	8	2418	44.0	29.5	30.0	F	-	60	643	44	497	52	519	52
70	S	5	9	2285	48.5	30.0	29.8	M	+	80	914	82	871	68	693	76
71	S	10	15	3260	50.0	33.0	33.5	M	+	78	1139	58	896	68	917	68
72	S	19	2947	51.0	33.0	31.5	F	+	36	903	40	844	36	704	37	
73	B	3	7	2371	46.0	30.0	29.4	F	-	80	914	82	871	68	693	76
74	O F	4	3501	50.0	33.5	32.0	F	-	40	645	36	654	40	728	38	

\* Symbols A T F Axis-traction forceps O F Outlet forceps S Spontaneous delivery B Breech delivery F "Forceps" + Yes - No

## RESULTS

Of the 74 infants described in Tables 1 and 2, records were obtained from 47 during sleep, and also from 3 others, not sleeping satisfactorily uniform records were secured. The results for these 50 infants are given in Table 1. The results for the remaining 24 infants are given in Table 2. The summaries found in the other tables and graphs and our conclusions are based upon the material in Table 1. The material in Table 2 is included for the sake of completeness and to show the difficulty of securing records of sleeping breathing and to indicate the great variability in the respiratory movements in the newborn child. A summary of the data dealing with the ages of the 50 subjects described in Table 1 is recorded in Table 3. The majority were tested during the first 48 hours of life.

TABLE 3

*Summary of data from table 1 to indicate the age of the [majority of] infants when tested*

Age at test	Number of Infants
During 1st 24 hours	27
During 2nd 24 hours	10
During 3rd 24 hours	5
4th to 11th days inclusive	8

TABLE 4

*Brief summaries of data taken from table 1*

	Weight grams	Length cm.	Sitting height cm.	Chest circum- ference at nipple line cm.	Respiratory rate per minute	Minute volume cc.	Mean tidal air cc.
Maximum	3989.0	53.5	35.2	35.0	116.0	1413.0	27.0
Minimum	2320.0	46.5	30.0	26.5	24.0	433.0	10.0
Average	3202.0	49.5	32.5	32.1	43.1	721.4	16.7

The maximum, minimum, and average figures for breathing rate, depth and minute volume are recorded in Table 4. The figures for rate and minute volume in this table are based upon the three samples (A, B, and C, Table 1) for each of the 50 infants. The mean tidal air is computed from the average rate and minute volume of the three samples from each infant. Both are based on 150 samples with the

group average computed differently. These observations indicate the wide variation which may be expected in the breathing rate and amount of ventilation in sleeping newborn infants, and also the variation in mean tidal air.

A frequency distribution of the breathing rate is found in Figure 4.

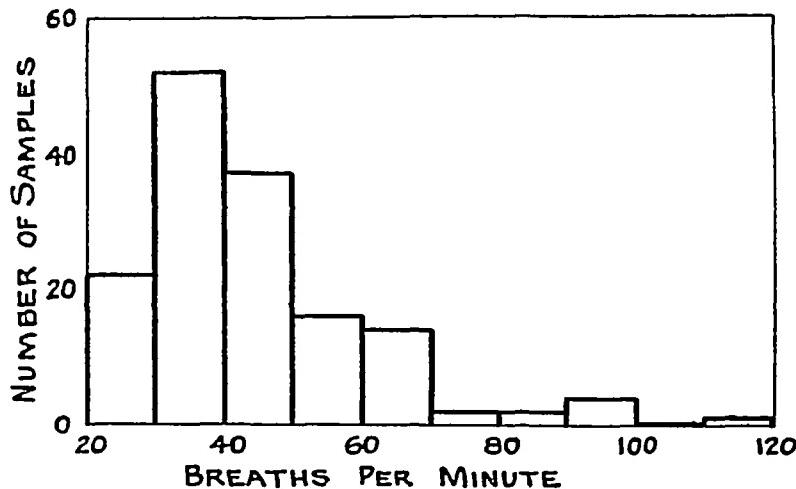


FIG. 4 DISTRIBUTION CURVE OF BREATHING FREQUENCY OF THE 50 INFANTS DESCRIBED IN TABLE 1

The abscissae indicate the number of breaths per minute. The ordinates record the number of breathing samples (3 for each infant). Note that the largest number of samples were registered by infants which breathed between 30 and 40 times per minute.

It is based on the 150 samples of the 50 infants described in Table 1. It will be evident that the largest group of infants breathed less than 40 per minute, though the average rate was 43.1 (Table 4). However there was a marked decrease in numbers of infants whose rate exceeded 50 per minute.

Figure 5 records the frequency distribution of the minute volume of air in the 50 sleeping infants. The largest group of infants breathed between 600 and 700 cc per minute while extremely few breathed more than 1000 cc. These observations, like the ones in Figure 4, are based upon the 150 samples from the 50 infants.

Figure 6 is a graphic record of the depth of the breathing, based on the mean tidal air for each infant (Table 1). From this chart it is

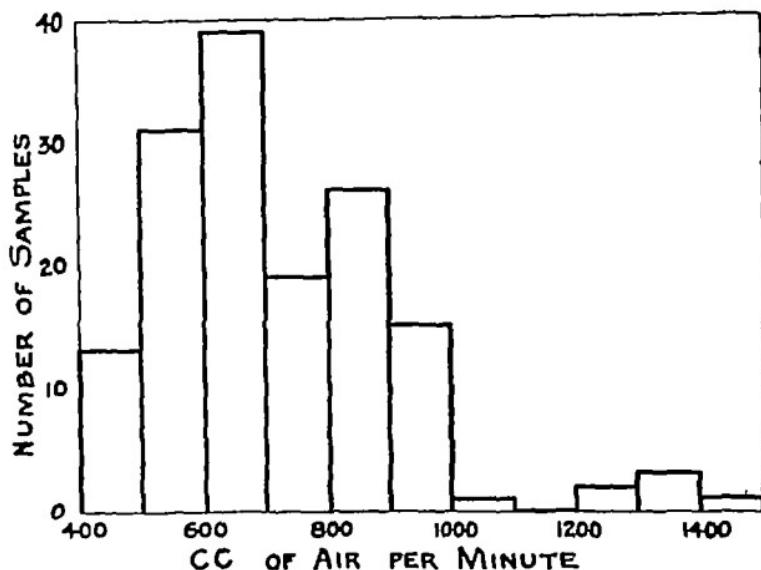


FIG. 5 DISTRIBUTION CURVE OF MINUTE VOLUME OF THE INFANTS DESCRIBED IN TABLE 1

The abscissae indicate the amount of air breathed per minute arranged in units of 100 cc the ordinates the number of samples (3 for each of 50 infants). Note that the majority of infants breathed less than 1000 cc per minute and that the largest single group less than 700 cc

evident that the greater number of the 50 infants breathed between 12 and 20 cc per breath

Two infants were tested twice daily (Table 5) Their records indi-

TABLE 5

Breathing data on 2 infants tested twice the same day both times asleep. Note the rate and minute volume variations in the case of infant A while the breathing depth remained constant. Figures represent averages of 3 samples

	Respiratory rate per minute	Mean tidal air cc	Minute volume cc
Infant A a.m	42	11	487
	64	11	704
Infant B a.m	44	14	642
	38	16	631

cate the variability in rate and minute volume which may be expected at different times within the same day, though the infants were sleeping and were presumably under identical conditions at both periods.

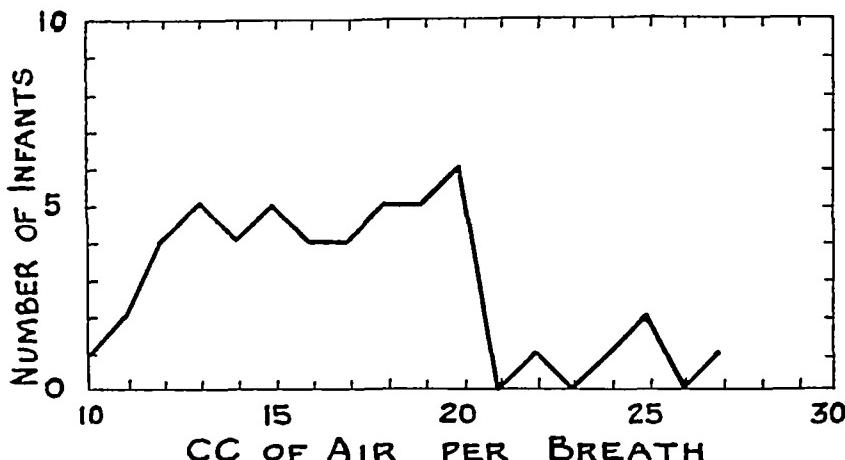


FIG. 6 DISTRIBUTION CURVE OF BREATHING DEPTH OF THE 50 INFANTS DESCRIBED IN TABLE 1

The abscissae indicate the size of the average breath (calculated from the average minute volume and the average rate, Table 1), the ordinates indicate the number of infants. Note that the great majority of the infants breathed between 12 and 20 cc, and that the numbers were fairly evenly distributed within these limits.

TABLE 6

*Breathing measurements of 4 sleeping infants taken on 3 successive days. Note wide variations in rates and in minute volumes. Figures represent averages of 3 samples.*

	Respiratory rate	Mean tidal air	Minute volume
Infant A, 1st day	per minute	cc	cc
	38	14	568
	60	13	783
Infant A, 2nd day	54	13	708
	46	14	671
	47	13	638
Infant A, 3rd day	32	17	545
	64	14	944
	36	17	639
Infant C, 1st day	42	16	707
	44	12	571
	44	11	511
Infant D, 1st day	32	14	471
Infant D, 2nd day			
Infant D, 3rd day			

Four infants were tested on each of three successive days (Table 6). These records like the preceding ones (Table 5) indicate wide variation in breathing activity and show the change that can be expected in the same infant from day to day. Both sets of observations show clearly that the ventilation is increased chiefly by an increase in rate, rather than by an increase in the depth of breathing. These infants (Tables 5 and 6) were measured under conditions similar to those maintained for the subjects of Table 1.

#### COMMENT

The observations recorded in Tables 1 and 2 are presented simply as a set of physiological measurements. An attempt to correlate the variations of the respiratory movements with other factors failed to yield deductions of any value. The study, however, indicates that the plethysmographic measurement of the breathing of the newborn is a practical and accurate method.

Even when sleeping the breathing of the newborn infants appears to vary within rather wide limits. This is observed chiefly in changes of rate, while the depth of the breathing remains relatively uniform. Thus breathing varies for reasons that as yet are not understood. In view of this marked variation, which is to be found in the same infant at different periods of the day, as well as on successive days, it is evident that in estimating the infant's physical condition its breathing rate should not be unduly stressed and this normal variation in breathing rate should be taken into consideration.

It has been stated that there is a gradual expansion of the lung in the newborn infant from day to day during the period immediately after birth. The present observations confirm this statement as will be seen by a glance at Table 6. It will be noted that the mean tidal air in infants B, C, and D increased with age. Our observations indicate that the degree of lung expansion cannot be estimated satisfactorily if only a few measurements of the rate of breathing and minute volume are taken into consideration, since these vary so widely from day to day.

The breathing rate as observed by us agrees fairly closely with rates noted by previous authors. The mean tidal air of the sleeping infant, however, appears to be less than that recorded by other observers. Also the minute volume measurements recorded by us are lower. The

## LITERATURE

Evidence in favor of the existence of anastomosis between the bronchial arborizations is contained in the work of Zimmermann (2), Hansemann (3), Merkel (4) and Schulze (5). By injecting masses intrabronchially and preparing corrosion specimens, they found that the pulmonary lobules were fused together at certain points. Others who have used similar methods with attention to this matter are Flint (6), Miller (7), Lagesse and d'Hardiviller (8) and Oppel (9), but they have not obtained such connections. The studies of Professor Joseph Marshall Flint are particularly noteworthy. In summarizing his results, he states that he found "at no period in the organogenesis of the lungs openings, or fenestrae, which suggest a communication between adjacent pulmonary units. They form, as we have seen, independently at the growing ends of the tree, and as they approximate each other, it is always possible to demonstrate the interlobular or interalveolar framework without interruptions suggestive of fenestrae offering a communication between adjacent alveoli. Furthermore, in all my cor-sions, many of which are complete enough to fill completely the alveoli pulmonalis and maintain the entire form of the lungs, no instance was found of an interalveolar communication." He points out, however, that the pulmonary lobules "may become compound by the loss of the interlobular septa and consequent confluence of several adjacent lobules." This work was done on pigs. Flint accounts for the appearances of interalveolar communication, which others obtained, as artifacts of extravasation of the injection masses, for high pressures are often necessary to drive the masses through the bronchi as far as the alveoli and delicate terminal passages are easily ruptured. The authoritative opinions of W. S. Miller (10) and Macklin (11) support this conception.

As long ago as 1733, Stephen Hales (12) showed that the lungs lose air from the surfaces when subjected to high pressures of inflation. He immersed a pair of dog's lungs in water in a bottle and then placed the whole within a negative pressure chamber, with the trachea connected by a tube to the outside. The pressure in the chamber was lowered and the lungs were gradually inflated. When the pressure reached  $-69\text{ cm H}_2\text{O}$  ( $-2\text{ in Hg}$ ) and the lungs were tensely distended, air began to escape in streams of bubbles from numerous points in the pleural surfaces. At pressures still lower,  $-240$  to  $-275\text{ cm H}_2\text{O}$  ( $-7$  to  $-8\text{ in Hg}$ ), the rate of escape of air was increased, but the number of points of exit in the pleura was not multiplied appreciably. This experiment has been repeated by Ewald and Korteb (13) in somewhat different form. Lungs were inflated under water simply by blowing into the trachea, and leakage was found to occur at pressures in the neighborhood of  $47\text{ cm H}_2\text{O}$  ( $35\text{ mm Hg}$ ). They also noticed the constancy in number of points of exit, and they added the observation that the minimum pressure of inflation required to produce leakage in a

given specimen was the same on repeated tests. Lungs in the living animal were likewise inflated and air was found to escape into the pleural cavity. Others (14, 15) have produced pneumothorax experimentally by the same procedure. Very forceful inflation was always necessary.

Bichat (16), in 1808, was the first to discover that convulsions and death were the result of inflating an animal's lungs at high pressures. Ewald and Robert saw the same thing. Since air was found in the blood vessels at autopsy these authors believed that the symptoms were due to air embolism and that the air entered into the pulmonary capillaries directly from the alveoli of the lungs. Joannides and Tsoulos (15) have recently studied the production of air embolism in this way. Also they are among several investigators who have produced interstitial emphysema of the lungs and mediastinum by that maneuver.

All of these sequelae of pulmonary hyperinflation are known to occur in man. Thus patients suffering crushing injuries of the chest without fracture of ribs often present pneumothorax or interstitial emphysema and either or both of these effects may even be the result of voluntary straining to blow or to lift. Instances are reported in which air has passed from the lungs into the blood stream as result of raised intrapulmonary pressure. Iversen (17) found quantities of air in the circulation of persons killed by hanging. Peltauf (18) described this in persons drowned and Lindblom (19), in new born infants which failed to respond to strenuously applied artificial respiration. The convulsive seizures that sometimes appear with paroxysms of whooping cough have been assigned by Neubürger (20) to cerebral air embolism, and Husler and Spatz (21) furnished support for this view by demonstrating at autopsy in such cases degenerative lesions of the brain precisely similar to those produced by air embolism.

The manner of escape of air from the lungs under these circumstances is not agreed upon completely. Most authors favor a theory of alveolar rupture indeed widely dilated and torn air sacs have been found in the lungs in some cases (22). But the observations mentioned as to the constancy in number of the points of exit of air from the pleura and the constancy of the minimum pressure of inflation required to produce escape of air by that path have led many to believe that the escape is through openings in the alveolar walls which exist normally and are open only when the alveoli are stretched by hyperinflation. They reason that if tears in the tissues were responsible repeated tests would show leakage at lower pressures than at first, and that the use of greater forces of inflation would multiply the number of openings.

Minute openings are well known to exist in the walls of the pulmonary alveoli in certain pathological states. In pneumonia strands of fibrin may be seen in histological preparations to pass from alveolus to alveolus through pin point apertures in the walls between and these are referred to as the pores of Kohn (23). They have been described also in pulmonary edema.

(10) although made out with difficulty because of the lack of fibrin to indicate their positions. The pores are believed by many authors (24, 25, 26, 27, 28, 29, 30) to be artifacts of fixation in histological preparation, or lesions. W. S. Miller (10) has described the process of their formation in pulmonary disease claiming that in each case the opening is formed in the interalveolar septum after necrosis of two epithelial cells lying opposite each other. Others (31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43) consider that the pores exist normally. Ogawa (44) gives good evidence of this in the results of his extensive studies of the minute anatomy of mammalian lungs. His histological methods permit the alveolar septa to be viewed in face as well as in cross section, and the pores are seen as oval openings in the surfaces, with smooth borders formed by the epithelial cells lining the alveoli. Ogawa states that the pores are readily distinguishable from artificial defects, for the latter present irregular and jagged borders and pass through, rather than between, the epithelial cells. The work was performed entirely upon normal lungs and includes a large variety of species.

#### EXPERIMENTAL MATERIALS AND METHODS

Lungs removed from the body were used for a part of the experiments, including specimens from man, dog, cat, rabbit, calf and pig. In the remainder of the experiments, lungs in the living animal were employed, and here dogs were the subject exclusively, because the endobronchial instruments at hand were adapted only to that species.

*Experiments in vitro.* The lungs were obtained and used immediately after death, with the exception of those from man, which were secured at autopsy from 8 to 20 hours after death. They were examined carefully for the presence of lesions and peculiarities of structure, and then a unit of one or more lobes was detached at a time and used for experimentation. Each lobe was prepared by tying a cannula into the stem bronchus or by similar treatment of one or more of the main bronchial branches. In doing the latter, it was necessary to dissect away the connective tissue at the hilus enough to expose and pass a ligature about each branch to be cannulated, and this was carried out with the utmost care to avoid injury to the parenchyma of the lobe. The majority of these experiments entailed injection of one cannula with air under delicately controlled grades of pressure. The apparatus for this consisted of a rubber tube connecting the bronchial cannula with a source of compressed air (tap from the main of the system of compressed air in the building) and two tubes branching from this,

one of which led to a manometer and the other of which ended shortly by immersion in a glass of water. The second of these branching tubes served to regulate the flow of air to the specimen. Sufficient current of air was turned on at the tap to be slightly in excess of that needed for inflation of the specimen, the excess air was allowed to escape constantly from the submerged tube, and by raising or lowering that tube in the water the flow to the specimen was regulated. While the injection was going on, the cannula of another bronchus was watched for the escape of air, and this was facilitated by immersing the free end of the cannula just under the surface of water in a separate dish. Some of the experiments required that the lung be expanded during the injection. The specimen was placed for this purpose in a negative pressure chamber with the cannulas extending to the outside, and the pressure within the chamber was lowered until the desired degree of expansion was obtained. The cannulas were then connected with the injecting apparatus and the test was made as described. The complete arrangement is illustrated in Figure 1.

*Experiments in vivo.* The dogs were prepared by hypodermic injection of morphine and atropine and were anesthetized by inhalation of ether. The neck was incised in the ventral mid line and the trachea was delivered and divided completely just below the larynx. The distal end of the trachea was fitted with a short glass sleeve of the same diameter, to hold wide the entrance for instrumentation and to control persistent oozing of blood. From this time on, the anesthesia was maintained by continuous intratracheal insufflation of ether-vapor and air. A bronchoscope was introduced through the tracheotomy opening, and one bronchus was chosen for cannulation the position and depth of its orifice being determined exactly. The bronchus chosen was either the main bronchus of the right lower lobe at a point just distal to the origin of the first branch of that lobe, or the bronchus supplying the right middle, lower and accessory lobes or the bronchus supplying the right lower and accessory lobes. Thus the first choice included one part of the bronchial tree of one lobe while the other two included the entire bronchial trees of two or three lobes. The bronchoscope was then removed and a long cannula was inserted in its place and fixed at the chosen point. A cannula was designed especially

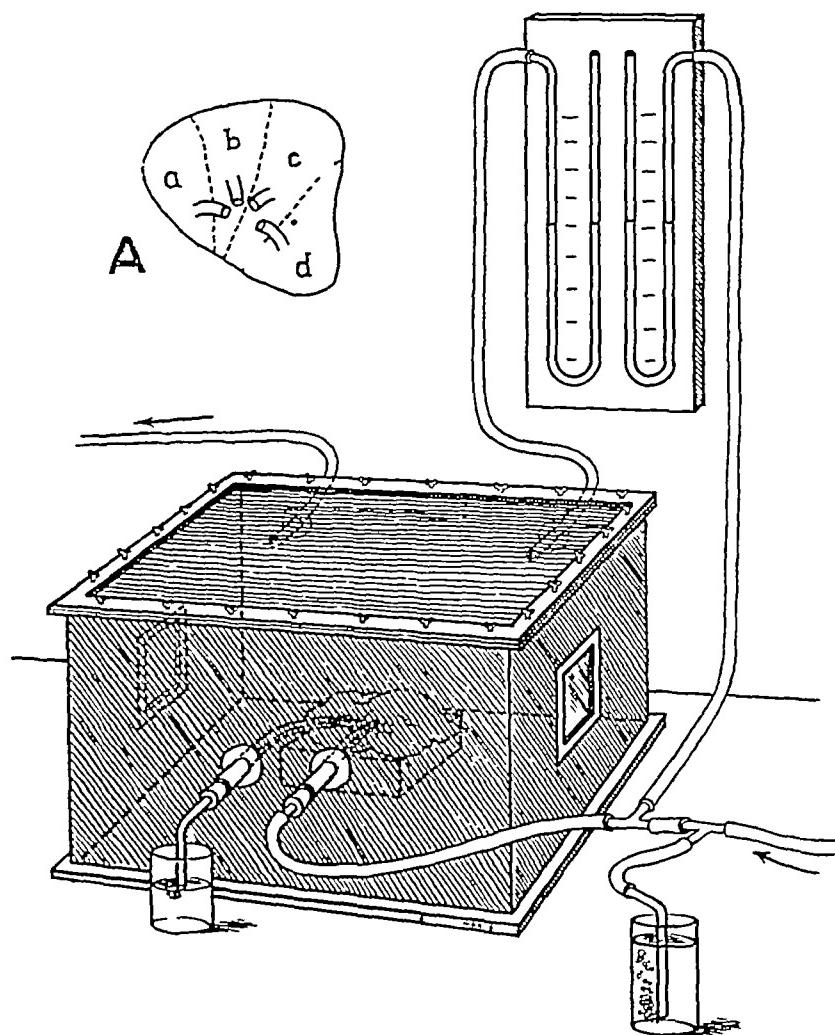


FIG 1 APPARATUS FOR MEASURING RESISTANCE TO COLLATERAL TRANSFER OF AIR IN THE EXCISED LUNGS

Center negative pressure chamber, containing one lung lobe. Two cannulas from the bronchi extend to the outside. Right, below system of tubes for injection of air. Left, below glass of water to detect escape of air. Right, above manometers. A diagram of lung lobe.

for this purpose (45)<sup>2</sup> (Fig. 2) One end could be dilated after introduction by revolving a cap at the other end The dilated end then presented a narrow ring of metal and rubber for selective attachment to the bronchial wall The animal was sacrificed and autopsied after the experiment in every instance, and the position and security of the cannula were determined It was invariably found that the attachment resisted vigorous twisting and pulling and that it showed no leakage of air under test with very high pressures In many experiments the cannula was provided with a valve for control of air respiration through it The valve was a bottle with two necks partly filled with water Two glass tubes entered the bottle through tight corks, one extending just beneath the surface of the water, and the other terminating above the water The cannula was connected by a rubber tube to the first of these when it was desired to permit only expiration through the cannula, and it was connected to the second when inspiration only was desired See Figure 3, A and B

*Manometric measurements* were made with water as medium since the pressures encountered were generally very small

Six objectives were dealt with in this work therefore the experiments will be presented in that number of sections For the sake of cogency each section will be treated separately by introducing it with statement of object and closing it with resume of deductions

## EXPERIMENTS

### Section I

*Object To test the air tightness of the pulmonary lobule seeking for transfer of air collaterally from its airways into those of adjacent lobules*

Experiments *in vitro* in man dog cat rabbit calf pig

*Protocol 1 Dog's lungs The bronchus of one lobule and that of one lobe injected in turn with air the inflation of the lobule and of the lobe and the paths taken by the air observed A dog's lungs were procured and examined The*

<sup>2</sup> Trial was made of the type of bronchial cannula employed by others (46 47 48) which has an inflatable rubber collar at one end to secure it in the bronchus This proved unsuitable for the purpose at hand for when the collar was inflated it elongated within the bronchial lumen and covered too great a length of the wall Also the rubber slipped easily and could not be depended upon to maintain a given position



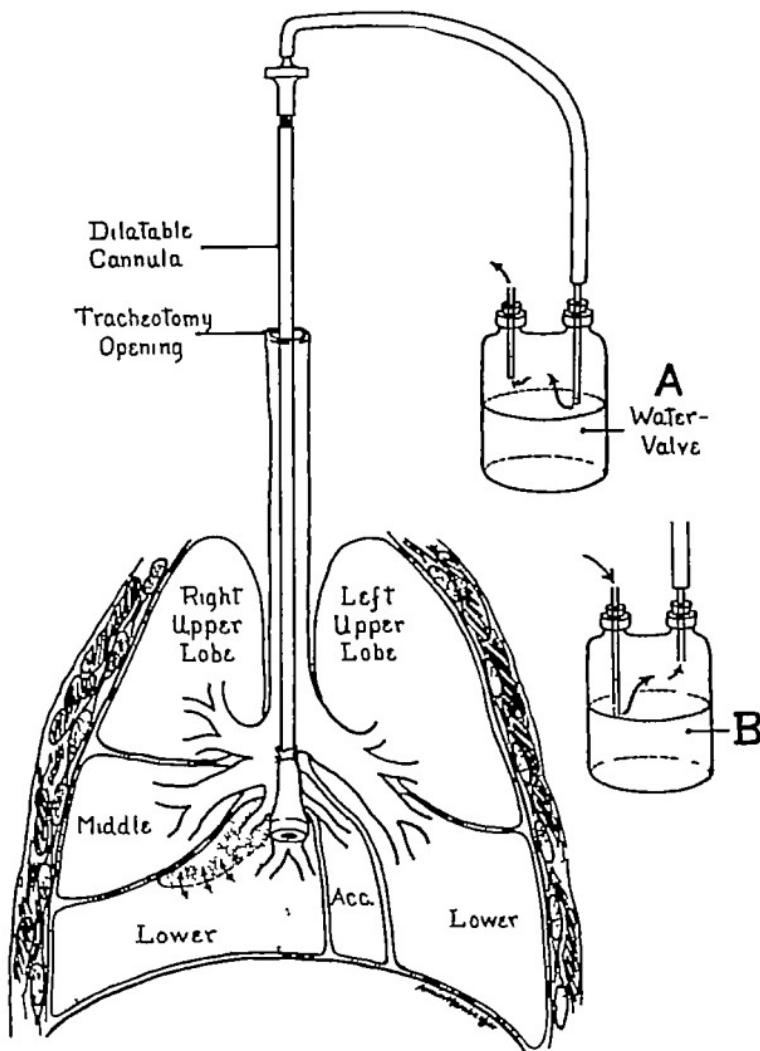


FIG 3 APPARATUS FOR DETECTING COLLATERAL RESPIRATION IN THE LIVING DOG

Center trachea and lungs with dilatable cannula fixed in one bronchus of the right lower lobe. Above connection to water valve A water valve, arranged to permit expiration only. B water valve arranged to permit inspiration only. Arrows path of collateral respiration between the obstructed part (lightly dotted) and the free part (densely dotted) of the cannulated lobe.

lobes were found to be entirely separated by fissures, except for very small areas of confluence near the hilus, and there they were held together by complete septa of loose connective tissue. The lobular markings on the surfaces were indistinct or quite lacking. The right lower lobe was selected first for testing and was detached from the specimen. One cannula was tied in the first branch from the stem bronchus and a second in the stem bronchus just distal to that point, so that the first cannula supplied a single major lobule and the second supplied the remainder of the lobe. The first cannula was connected to the apparatus for injection of air, and the second was submerged in water. A slow current of air was then turned on and regulated so as to inflate the lobule very gradually. The lobule was seen to enlarge symmetrically, and it soon showed scattered patches of cortical inflation. When the expansion was about three-fourths normal, the progress halted and air began to escape from the second cannula in a continuous stream of bubbles without there having been the slightest appearance of inflation of the other portion of the lobe. The escape of air was observed for a few minutes and no change was noted, and then the rate of injection was made steadily to increase. As a result of this the lobule expanded more and more and the flow of air from the second cannula became greater and greater. When the expansion had reached a degree that was about normal, the rate of injection was reduced and regulated so as to maintain that state, and the specimen was submerged in water. No air escaped from the pleura. Then the specimen was taken from the water and the second cannula was closed. The portion of the lobe supplied by it began immediately to inflate and continued to do so until it was fully distended. It presented the same appearances of inflation as were shown by the single lobule, i.e., symmetrical expansion. At no time was interstitial extravasation of air seen in the plane of fusion of the two divisions of the lobe or at other points. The first cannula was then closed, stopping the air current, and the second cannula was opened. The lobe collapsed rapidly as a whole, but when air had ceased to escape the lobule supplied by the first cannula remained very slightly more inflated than the rest of the lobe. The lobe was discarded, and the right upper and middle lobes were detached from the specimen for testing. They were allowed to remain connected together, for they presented a maximum degree of confluence (over an area of about 1 sq cm). The stem bronchus of each lobe was fitted with a cannula. That of the right upper lobe was connected with the apparatus and injected with air, while the cannula of the other lobe was submerged in water and watched for the escape of air, just as in the preceding experiment. The inflation of the first lobe was carried far beyond the normal degree of expansion and to a point when air escaped audibly from the pleural surfaces, but no air bubbled from the second cannula.

These experiments were repeated with many other specimens of dogs' lungs and the results were always similar. The lungs of cats were used in

the same way. Their gross anatomical characteristics and their behavior to lobular and lobar inflation were quite similar to those of the dogs lungs and do not require separate description.

*Protocol 2 Dog's lungs—one atelectatic lobe* The bronchus of one lobule injected with air the inflation of the lobule and the path taken by the air observed roentgenographically. The accessory lobe of a dog's lungs was obtained. It had been rendered atelectatic and was shrunken and uniformly airless.<sup>3</sup> The lobe was bilobular suggesting somewhat the shape of a butterfly and the stem bronchus bifurcated at the hilus into branches of equal size for the two wing like major lobules. A cannula was tied into each of the two branches and they were connected with apparatus for injection of air. A roentgenogram was then taken of the specimen and adjacent connections. This is reproduced in Figure 4 at A and shows the lobe as a shadow of homogeneous consistency. The cannula for injection is represented at a the exhaust tube for controlling the injection is shown at b and the second cannula is at c with its free end submerged in water in a small dish at d. Injection was begun and one lobule was inflated gradually until air began to escape from the cannula of the other and with the air continuously bubbling through the water a second roentgenogram was taken. See Figure 4 B. Here at e the aerated portion of the lobe is represented as an area of rarefaction including the greater part of the first lobule and a narrow zone at the border of the second. At f appears a circular area of rarefaction betraying the escape of air through the water. The second cannula was then closed by turning a stop-cock, to retain the air entirely in the specimen and the progress of inflation that ensued was followed in a series of roentgenograms. See Figure 4 C D E and F. This shows that the air spread to the bronchi of the second lobule, at g and to the parenchyma of both lobules until all parts of the lobe were filled with air.

This experiment was performed with one other specimen and similar results were obtained.

*Protocol 3 Lungs of man* The bronchi of several lobules injected in turn with air the inflation of the lobules and the paths taken by the air observed. The left lung was obtained from a young man who had died a few minutes after receiving abdominal and cerebral injuries. The two lobes were confluent over about one half of their opposed surfaces and in the plane of confluence was a complete septum of loosely packed connective tissue. Lobular markings were indistinct for the most part. The lobes were allowed to remain attached together and they were prepared for testing by cannulating the three main branches of the stem bronchus of each lobe. Two branches were chosen for use at first the lobules of which lay adjacent and

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<sup>3</sup> The atelectasis was produced by obstructing the stem bronchus of the lobe with wax 3 days before sacrifice (49 50). The wax was removed at autopsy before the lobe was used.

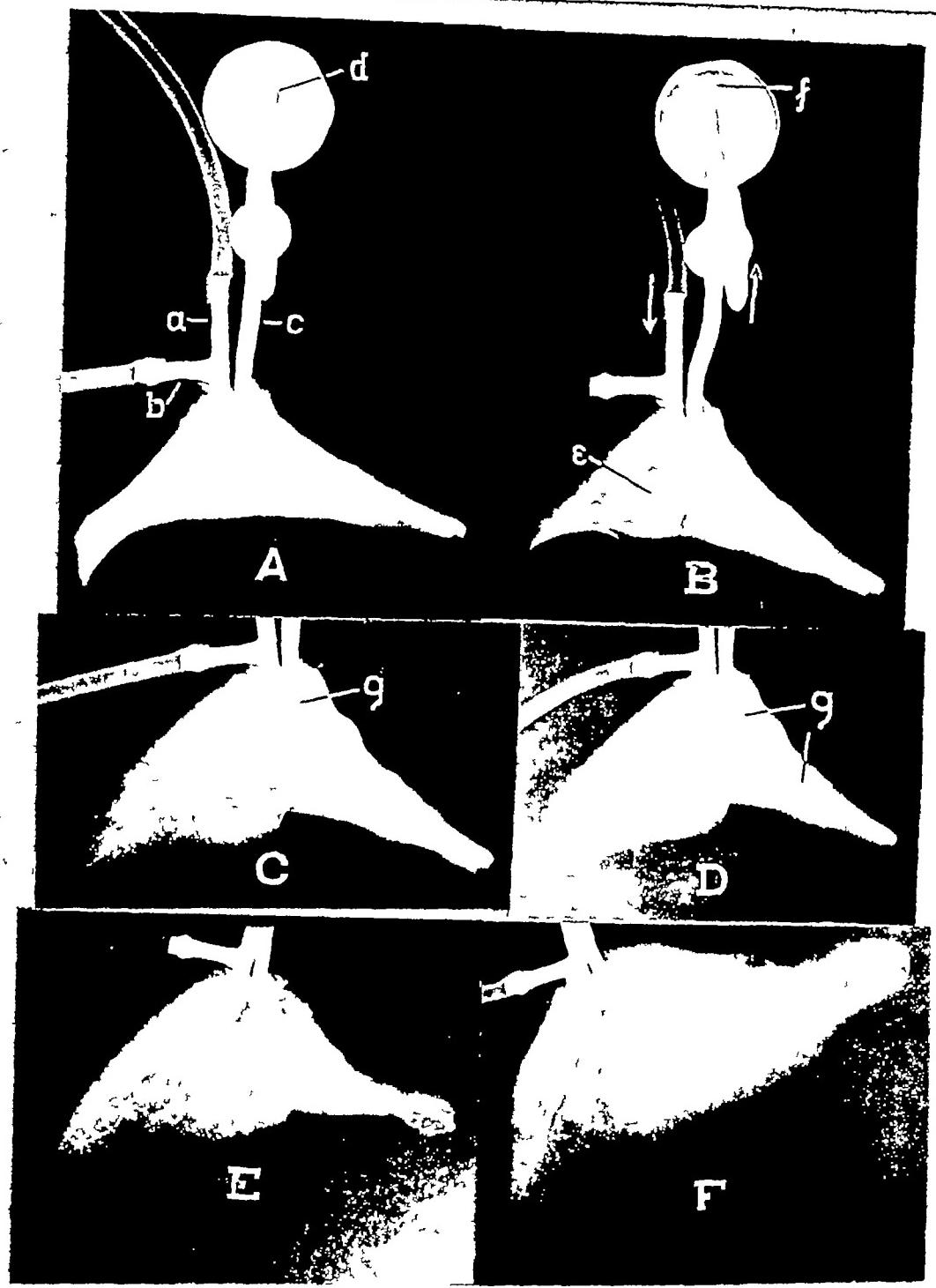


FIG 4 SERIES OF ROENTGENOGRAMS OF A LOBE OF DOG'S LUNGS, MADE WHILE AIR WAS BEING INJECTED INTO ONE BRONCHIAL BRANCH

A lobe fully collapsed B-E stages of inflation, showing collateral transfer of air from one major lobule (*e*) to the second (*g*), with escape (*f*) from the bronchus of the latter F lobe fully inflated

the cannulas of the others were closed. Connections were made and air was injected into one while the other was watched for escape of air. The lobule of the first became inflated to about one fourth normal expansion when air began to flow from the cannula of the second. The flow was continuous and kept pace with the injection, the expansion of the lobule remaining constant. The rate of injection was then increased. The lobule expanded farther and farther, and when the expansion had reached about a normal degree the escape of air from the second cannula had increased to an exceedingly rapid flow. There was no loss of air from the pleural surfaces nor appearance of interstitial emphysema. The cannulas were then disconnected from the apparatus and after the air had escaped from the lobule they were closed. The test was applied to two other lobules which lay adjacent to each other and in the other lobe. The result was the same. Again, a pair of lobules was chosen which lay adjacent to each other but in different lobes (with the interlobar septum between) and the test was applied. This time the inflation of one lobule had to be carried distinctly beyond normal before air escaped from the cannula of the other.

Another normal human lung was tested in this way. The interlobar fissure was deeper in this case and the area of confluence was proportionately less extensive. The plane of contact was occupied by a complete septum of connective tissue as in the other specimen. The results were similar in every respect, except that when two lobules were tested which lay adjacent to each other and in different lobes, there was no transfer of air even when inflation of one was carried far beyond normal with loss of air from the pleural surfaces and interstitial extravasation.

*Protocol 4 Rabbit's lungs* The stem bronchus of two lobes injected in turn with air, the inflation of the lobes and the paths taken by the air observed. The lungs were secured from a large rabbit. The lobes were found to be widely confluent and the interlobar septa were thin and incomplete. Lobular markings were lacking. The stem bronchi were so imbedded in the lung that it proved impossible to expose the first branches for cannulation without injuring the parenchyma so the experiment had to be limited to dealing with whole lobes. The middle and lower lobes on the right were selected for the first test and, leaving them attached to the lungs the stem bronchus of each was fitted with a cannula. The cannula of the middle lobe was connected to the apparatus for injection and that of the lower lobe was closed. Injection was begun. The middle lobe inflated to a point which represented about one half normal expansion when the other lobe began to expand and then the process continued in both to about three fourths normal expansion. At this point it ceased and air could be heard to escape from the pleural surfaces of both lobes. (Pressure of injection 20 cm H<sub>2</sub>O.) The left upper and lower lobes were tested in the same manner and similar results were obtained. In neither case was interstitial emphysema seen.

*Protocol 5 Calf's lungs* The bronchi of several lobules injected in turn

*with air, the inflation of the lobules and the paths taken by the air observed.*

The lungs of a calf were secured. The lobes were found to be separated almost entirely by fissures, and thick septa occupied the small planes of interlobar confluence that existed. The lobular markings were very pronounced. By dissecting with blunt instruments between the lobules, it was discovered that the septa lying in that position were much heavier than those seen in the lungs of the other animals examined, and that between the major lobules they extended throughout, from hilus to periphery. The septa between the minor lobules separated only the peripheral parts, and in the deeper parts the lobules were intimately fused together. The right lower lobe was first chosen for testing and was detached from the lungs. The three main branches of the stem bronchus were fitted with cannulas. Two adjacent branches were used first, and the other was closed. One of the pair was connected with the apparatus for injection, and its lobule was inflated. The expansion reached an approximately normal degree without any air escaping from the cannula of the second lobule. Inflation was carried further, and soon air was heard plainly to leak from the pleural surfaces. With more inflation, air began to extravasate into the septum between the lobules, to split the two lobules apart, and to form blebs under the pleura. Still no air passed from the cannula of the second lobule. The apparatus was now disconnected and the lobule was allowed to collapse. The cannula of the third lobule was connected for injection, that of the second was submerged in water to be watched for escape of air, and that of the first was closed. The second and third lobules were adjacent. The results of the injection were the same as before. Attempts were then made to apply the test to minor lobules, but the bronchi supplying them were too small to be cannulated. The right upper lobe was next taken from the specimen of lungs and tested, and the results were no different than before.

The lungs of a pig were obtained and examined. They presented interlobar and interlobular septa as heavy and extensive as those found in the lungs of the calf. The results of experiments with inflation of lobules were very similar to those described in the protocol above.

#### *Deductions*

1. The air passages of lobular divisions of excised lungs are not airtight at the periphery. When the inflation of one lobule alone is increased, the air may escape by one or more of three courses besides that through the bronchus of the lobule, viz., into the air passages of neighboring lobules, through the pleura, and into the interstitial tissues. The first of these courses is undertaken when the inflation of the lobule is increased moderately and within normal limits, while the other two courses are assumed only with over-inflation of moderate

or extreme degrees. An exception to this occurs with rabbit's lungs, for the second course is undertaken at degrees of inflation only slightly greater than those at which the first course is assumed. The first course, namely collateral transfer between lobules, depends also upon intimate interlobular fusion, and this circumstance varies with the species of animal and with the regions of the lungs as follows. In man, dog and cat, lobules in the same lobe are intimately fused, and lobes are separated completely by fissures and thick septa, and in these species air passes freely from lobule to lobule when both of them are contained in the same lobe but not when they are contained in different lobes and lie adjacent. The rabbit presents intimate fusion between lobules and between lobes, and air passes freely from lobule to adjacent lobule whether the lobules lie in the same or in adjacent lobes. The calf and pig have thick septa and fissures which completely separate the lobes, thick septa which completely separate the major lobules, and thinner septa which only partly separate the minor lobules, and in these species air fails to pass between major lobules in any position. Passage between minor lobules could not be tested for but that there was provision for such passage was evident from the intimate interlobular fusion that existed.

2 Collateral transfer of air between the bronchial passages of adjacent pulmonary lobules probably occurs by way of the fine arborizations at the plane of interlobular fusion, since it occurs only when the air extends to the periphery. Moreover, the transfer seems to be direct and without traversing connective tissues because, first, interstitial emphysema does not appear in the region where the transfer occurs; second wherever the transfer fails and interstitial emphysema develops from over inflation the transfer is not initiated thereby; and, third, the pattern of expansion produced in one lobule by transfer of air from another is symmetrical and exactly the same as that of a lobule which is inflated by injection into the bronchial tree.

### Section 2

*Object To test the air tightness of the pulmonary lobule, seeking for transfer of air collaterally into its bronchial arborizations from those of adjacent lobules*

*Experiments *in vivo* in the dog*

*Protocol 6 Living dog's lungs The bronchus of a division of lobules in one*

*lobe and that of three entire lobes aspirated in turn, to exhaust the air from those parts of the lungs, the yield of air measured and compared with the estimated capacities of those parts for air, the effects of the aspiration upon intrabronchial pressures observed* A dog was anesthetized and tracheotomized The dilatable cannula was introduced through the tracheotomy opening and fixed in the bronchial tree of the right lower lobe, at a point just distal to the origin of the first branch of the lobe The cannula was connected to a manometer and the intrabronchial pressures were read (respirations light) They were inspiration,  $-22\text{ cm H}_2\text{O}$ , and expiration,  $25\text{ cm H}_2\text{O}$  A syringe was then connected in place of the manometer, and air was aspirated from the cannula at the rate of 100 cc per minute The yield of air was free at all times, and when 500 cc, which was at least 5 times the amount of air normally contained in the part of the lobe cannulated, had been removed without indication of exhausting the supply, aspiration was stopped and the syringe was replaced by the manometer The pressures were inspiration,  $-20\text{ cm H}_2\text{O}$ , and expiration,  $25\text{ cm H}_2\text{O}$  Next, the cannula was loosened from the bronchus, withdrawn slightly and fixed again at a point in the right primary bronchus, so as to include in the cannulation the whole of the middle, lower and accessory lobes The syringe was connected with the cannula once more and air was aspirated at the rate of 100 cc per minute With removal of 125 cc resistance to aspiration increased perceptibly and continued to do so as more air was removed, until at 200 cc, which was less than the amount normally contained in the three lobes and about equal to the amount which they would be expected to yield on collapsing, no more could be withdrawn The intrabronchial pressure at that time proved far too low to be measured by the manometer

This experiment was repeated in several other dogs and similar results were obtained The observation was added that aspiration of small amounts of air, as small as 3 cc, from one or more whole lobes resulted in distinct and lasting depression of the intrabronchial pressures This effect was always in contrast to that of aspiration from lobular divisions of single lobes, where removal of air in any quantity failed to change the pressures

#### *Deductions*

3 The air passages of lobular divisions of the lungs in living dogs are not air-tight at the periphery When the inflation of one lobular division alone is decreased air may enter freely by another way than that of the bronchus of the lobule The portal of entrance is probably the collateral passage, already referred to, which connects the airways of adjacent lobules The collateral transfer occurs so freely as completely to restore the inflation of the lobular division in question

4 Collateral transfer of air does not take place from lobule to lobule

when the lobules lie adjacent and in different lobes of the lungs of living dogs

### Section 3

*Object To test for collateral respiration on the part of the pulmonary lobule with obstructed bronchus*

#### Experiments *in vivo* in the dog

*Protocol 7 Living dog's lungs* The bronchus of a division of lobules in one lobe and that of two entire lobes obstructed in turn with a valve to allow only expiration from those parts of the lungs, the total amounts of air expired past the valve compared to the estimated capacities of those parts for air, the expired air analyzed the valve reversed and the total amounts of air inspired past the valve compared to the estimated tidal air of those parts A dog was anesthetized and tracheotomized The dilatable cannula was introduced and fixed in the bronchial tree of the right lower lobe, at a point just distal to the origin of the first branch of the lobe. The cannula was connected to the submerged tube of the water valve, Figure 3 A Now with the first expiration air bubbled freely from the submerged tube and with the following inspiration bubbling ceased and a column of water was sucked up in the tube about 5 cm The second expiration was accompanied by a like discharge of air and the inspiration produced the same elevation of water and these effects accompanied each respiratory cycle throughout an half hour period of observation The total volume of air which escaped during that period was obviously many times that of the capacity of the division of the lobe from which it came The bronchial cannula was then disconnected from the valve and free respiration was permitted to take place through the cannula for a few minutes The cannula was reconnected to the valve but this time to the elevated tube, Figure 3 B Now, with the first expiration a column of water rose about 5 cm in the submerged tube of the valve and with the following inspiration air bubbled freely from that tube The second and every other respiration in an half hour period of observation produced the same effects The dog gave no sign of respiratory embarrassment. The total volume of air which entered the cannula was obviously enormously greater than the capacity of the cannulated division of the lobe to receive air Next the cannula was disconnected from the valve and loosened from the bronchus It was withdrawn in the lung slightly and fixed again at a point in the primary bronchus so as to include in the cannulation the whole of the lower and accessory lobes The cannula was connected to the submerged tube of the valve With the first expiration air bubbled freely from the tube and with inspiration water rose about 5 cm in the tube The second expiration produced distinctly less bubbling the third and fourth produced still less and after that no more air was expelled past the valve with the exception that one or two times when expiration was unusually forceful a bubble escaped The inspiratory elevation of the water in the tube in

creased with each respiration and, after the fourth or fifth, remained at about 8 cm. The total volume of air which had escaped at the valve was evidently no more than the amount of tidal air that the cannulated division of the lobe might be supposed to contain. The dog was sacrificed, and the position and security of the attachment of the cannula were verified.

This experiment, with the order of the maneuvers varied, was performed in many dogs, and the results were always similar to these. In one case, the air expired from a division of the lower lobe over a period of 3 hours was measured and found to amount to 4,000 cc., and in another case the amount was 2,700 cc. in 1½ hours. On the other hand, the quantity of air expired from a cannulated unit of two or three whole lobes was never greater than the volume of tidal air which it was reasonable to suppose that portion of the lungs normally possessed.

#### *Deductions*

5 After obstruction of the bronchus of a division of lobules in one pulmonary lobe in living dogs, that division may inspire and expire spontaneously and freely by another channel and maintain its inflation. The channel is probably the collateral passage, already referred to, which connects the airways of adjacent lobules. This function may be termed collateral respiration.

6 The fact that collateral respiration occurs during quiet breathing spontaneously after bronchial obstruction characterizes it as a natural function and eliminates the possibility of its occurrence as an artifact from rupture of air passages.

7 Collateral respiration does not take place between lobules which lie adjacent in different lobes (dog).

#### *Section 4*

*Object To measure the resistance offered to collateral transfer of air between pulmonary lobules*

Experiments *in vitro* and *in vivo*, in man, dog and cat.

*Protocol 8 Lungs of man One lobe expanded in a negative pressure chamber, the bronchi of several lobules injected in turn with air, the force of inflation necessary to transfer air collaterally between lobules measured.* The upper lobe was obtained from the lungs of a young woman who had died acutely from internal abdominal injuries. The stem bronchus was found to divide into four branches outside the lobe, and the lobules which these supplied were arranged side by side as shown in the diagram, Figure 1, A, lettered *a, b, c, d*. All four were fitted with cannulas. The specimen was placed in a negative pressure chamber, with cannulas *b* and *c* extending to

the outside and cannulas *a* and *d* closed. The lobe was then inflated to about normal expansion. When equilibrium was established (pressure of -10 cm H<sub>2</sub>O in the chamber) cannula *b* was connected with the necessary apparatus and injected with air, while cannula *c* was submerged in water and watched for the escape of air. Figure 1. The pressure of injection was 3.6 cm H<sub>2</sub>O at the time that escape began and at pressures very slightly higher than this the escape was exceedingly free. With the flow thus established the pressure of injection was lowered and it was found that the flow continued until a pressure of 2 cm H<sub>2</sub>O was reached. The first reading was called 'initiation pressure' and the second 'minimum' pressure. Cannula *b* was then closed and replaced by cannula *d* and the latter was injected with air and cannula *c* was observed. (Pressure of -10 cm H<sub>2</sub>O in the chamber.) The initiation pressure was found to be 1.5 cm H<sub>2</sub>O and the 'minimum' pressure 1.2 cm H<sub>2</sub>O. Again cannula *d* was closed and exchanged for cannula *a*. (Pressure of -10 cm H<sub>2</sub>O in the chamber.) Cannula *a* was injected and *b* observed. The initiation pressure was 4.0 cm H<sub>2</sub>O and the 'minimum' pressure 2.8 cm H<sub>2</sub>O.

This experiment was performed also with lung lobes from two dogs and one cat. Each lobe had two cannulas one supplying a single lobule and the other the remaining lobules. The left lower lobe of one dog's lungs gave readings of 3.0 cm H<sub>2</sub>O for initiation pressure and 1.4 cm H<sub>2</sub>O for 'minimum' pressure. The left upper lobe of another gave 4.0 cm H<sub>2</sub>O for 'initiation' pressure and 1.6 cm H<sub>2</sub>O for 'minimum' pressure. The right lower lobe of the cat gave 1.6 cm H<sub>2</sub>O for 'minimum' pressure.

The same experiment, without the chamber and with the lungs collapsed as from autopsy was carried out with one specimen from man and with specimens from several dogs. The readings were consistently somewhat higher than those which have been given for expanded specimens (Table 1).

TABLE 1

*Minimum pressure of inflation required for collateral transfer of air between pulmonary lobules in vitro*

Species	Lobe collapsed cm H <sub>2</sub> O	Lobe expanded cm H <sub>2</sub> O
Man	7.5	2.0
	7.5	1.2
	5.5	
Dog	10.0	1.4
	10.0	1.6
Cat		1.6

*Protocol 9 Living dog's lungs. The bronchus of a division of lobules in one lobe obstructed with a valve to allow only expiration for that part of the lungs the pressures in the obstructed and free bronchi measured at inspiration the*

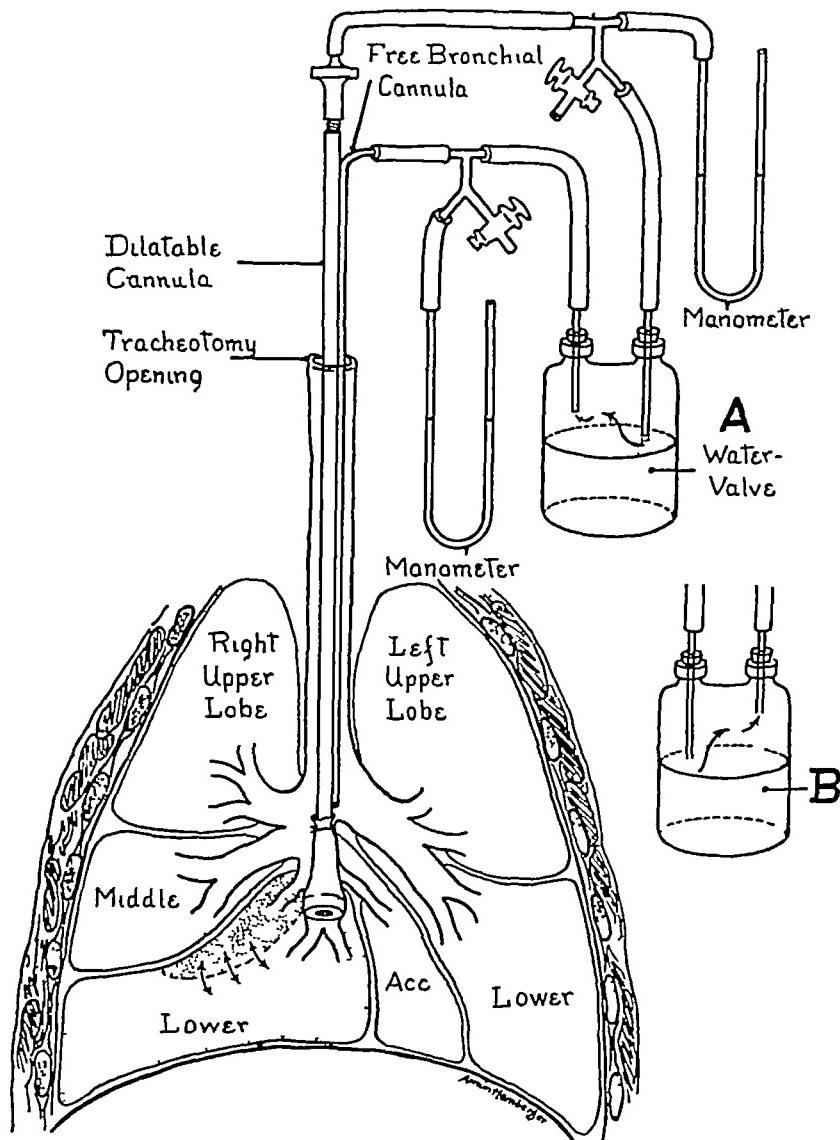


FIG 5 APPARATUS FOR MEASURING RESISTANCE TO COLLATERAL RESPIRATION

To the apparatus of Figure 3 have been added one free bronchial cannula with connections to the water-valve, and two manometers

valve reversed, the pressures in the obstructed and free bronchi measured at expiration the forces producing collateral respiration calculated. A dog was anesthetized and tracheotomized. The dilatable cannula was introduced through the tracheotomy opening and fixed in the bronchial tree of the right lower lobe at a point just distal to the origin of the first branch of the lobe. The cannula was connected to the submerged tube of the water valve and to a manometer. A long slender tube was then passed beside the bronchial cannula through the trachea to the primary bronchus and its end was brought to lie freely within the lumen just proximal to the point of attachment of the cannula. This tube was connected to the elevated tube of the water valve and to a second manometer. Figure 5, A. Now, at each expiration air bubbled past the valve (from the cannulated part of the lungs to the primary bronchus) and at each inspiration water rose a short distance in the submerged tube of the valve. This action was observed for about one half hour and noted to be persistent. The manometers were read from time to time and the readings were found to be stationary. Representative readings were 1.4 cm H<sub>2</sub>O and 0.4 cm H<sub>2</sub>O at expiration and -1.8 cm H<sub>2</sub>O and -0.6 cm H<sub>2</sub>O at inspiration. The first of each of these pairs of readings was from the manometer attached to the dilatable cannula and represented the pressure within the obstructed bronchi, and the second of each was from the manometer of the free cannula and gave the pressure within the unobstructed bronchi. The difference was taken between the two readings at inspiration which is 1.2 cm H<sub>2</sub>O, and this was interpreted to be the predominance of pressure in the unobstructed as compared to the obstructed bronchi effecting the collateral transfer of air into the obstructed division of the lobe. Next, the water valve was reversed. Figure 5, B. At each inspiration air bubbled past the valve (from the primary bronchus to the cannulated part of the lungs) and with expiration water rose a few centimeters into the submerged tube of the valve. This was observed for one half hour noted to persist, and the manometers were read from time to time. Representative readings were -1.2 cm H<sub>2</sub>O and -0.2 cm H<sub>2</sub>O at inspiration and 1.0 cm H<sub>2</sub>O and 0.5 cm H<sub>2</sub>O at expiration. The order in each pair of these readings is the same as before. The difference between the two at expiration was taken, which is 0.5 cm H<sub>2</sub>O and this was interpreted to be the predominance of pressure in the obstructed as compared to the unobstructed bronchi effecting the collateral transfer of air out of the obstructed division of the lobe. (Table 2.)

This experiment was repeated in its essential features on many other dogs and the values obtained were the same or very similar to those when the animals were breathing lightly as in this experiment. With deeper breathing the values were higher that is the predominances of pressure effecting collateral respiration were higher often many times the values quoted.

TABLE 2

*Intrabronchial pressures produced in breathing after obstruction of a lobular bronchus in vitro*

Position	At inspiration cm H <sub>2</sub> O	At expiration cm H <sub>2</sub> O
In obstructed bronchi	-1.8	1.0
In free bronchi	-0.6	0.5
Difference	1.2	0.5
(Force producing collateral inspiration)		(Force producing collateral expiration)

#### *Deductions*

8 The resistance offered to collateral transfer of air between pulmonary lobules *in vitro* is very small, particularly when the specimen is somewhat expanded during examination. It may be overcome by force as small as the weight of 1 cc of water.

9 After obstruction of the bronchus of a pulmonary lobule *in vivo*, the pressures of breathing in the obstructed air passages fluctuate more widely than do the pressures in the free passages. Thus, during inspiration, the pressures in the obstructed passages fall below those in the free passages, and during expiration the former rise above the latter. These differences, or predominances, are proportional with the depth of breathing, being as low as 0.5 cm H<sub>2</sub>O pressure in very light breathing and very much greater in forceful breathing. These are evidently the forces which effect the transfer of air in collateral respiration. It may be said, therefore, that the resistance offered to collateral transfer of air between pulmonary lobules *in vivo* is exceedingly small, smaller than *in vitro*.

10 Air may be transferred collaterally between pulmonary lobules in the same lobe, even when the two concerned are separated by another lobule with obstructed bronchus. Under these circumstances, the resistance to the passages of air is somewhat increased but remains within the limits of the forces which act during quiet breathing to effect collateral respiration.

#### *Section 5*

*Object To characterize the connections by which air is transferred collaterally between pulmonary lobules*

*Experiments in vitro and in vivo, in the dog*

*Protocol 10 Dog's lungs—one lobe* The bronchus of one lobule instilled with colored water (methylene blue solution) the path taken by the water observed The right lower lobe of a dog's lungs was obtained and prepared by tying a cannula in the stem bronchus and a second in the first bronchial branch The cannulas were then held in the vertical position suspending the specimen from their lower ends and a 0.5 per cent aqueous solution of methylene blue was instilled drop by drop into the cannula of the bronchial branch until no more would immediately enter The lobule supplied by that branch became deeply stained The cannula was then closed and the specimen was laid aside for one half hour No change was noted in the external appearance of the specimen during that time but frothy fluid stained deep blue was found in the cannula of the stem bronchus and in the larger bronchi of the part of the lobe which it supplied Cross section of the lobe showed the parenchyma of the separately cannulated lobule sodden with fluid deep blue and sharply demarcated from the parenchyma of the rest of the lobe which was unstained

In other experiments of this sort the stain was injected with 15 to 30 cm H<sub>2</sub>O pressure In addition to immediate staining of the injected lobule, as before these specimens showed points and areas of staining in the surfaces of the rest of the lobe within one or two minutes Cross section of the lobe showed solid infiltration of the injected lobule with stain and patches of staining in the parenchyma of the other parts At the center of the patches were bronchioles filled with the stain The line of demarcation of the injected lobule was sharp and there was no appearance of direct extravasation from its borders When injection was carried on a few minutes longer than this, the lobe became colored throughout with the stain

The experiment of protocol 10 was repeated once using a colloidal solution (5 per cent argyrol solution) for injection Precisely the same results were obtained

*Protocol 11 Dog's lungs—one lobe* The bronchus of one lobule injected with water containing grossly discernible solid particles (bismuth subnitrate crystals) in suspension the path taken by the particles observed roentgenographically The left lower lobe of a dog's lungs was obtained and prepared by tying a cannula in the first branch of the stem bronchus An aqueous 20 per cent suspension of bismuth subnitrate (the most finely powdered commercial preparation) was injected with 10 cm H<sub>2</sub>O pressure into the cannula and the pressure was maintained for about 15 minutes A roentgenogram was then taken of the specimen The pressure was raised to about 50 cm H<sub>2</sub>O and a second roentgenogram was made Both negatives showed the injected lobule to be saturated and distended with the opaque mass and the rest of the lobe to be free from it.

*Protocol 12 Dog's lungs—one lobe* The lobe expanded in a negative pressure chamber the bronchus of one lobule instilled with water containing microscopic solid particles (granules of India ink) in suspension, the path

taken by the particles observed. The right lower lobe of a dog's lungs was obtained and prepared by fixing one cannula in the stem bronchus and another in the first bronchial branch. The specimen was then placed in the negative pressure chamber with the cannulas extending to the outside and a moderate degree of expansion was produced by lowering the pressure in the chamber to  $-10$  cm H<sub>2</sub>O. The chamber was then tilted onto its side, so as to bring the cannulas to a vertical position suspending the specimen from their lower ends. Three cc of india ink, diluted with water to 50 per cent strength, was instilled into the cannula of the bronchial branch. The pressure in the chamber was then made to fluctuate between 0 and  $-10$  cm H<sub>2</sub>O pressure four or five times, to collapse and expand the lobe alternately. Examination then showed that the cannula of the stem bronchus and the large bronchi of the part of the lobe which it supplied contained black frothy fluid.

This experiment was repeated in three other specimens, and the same results were obtained. Once, india ink was instilled into a separately cannulated lobule without the use of expansion, and the specimen was allowed to remain for 3 hours before examination. The instilled lobule was deeply and completely blackened, but no trace of ink could be found in the other parts.

*Protocol 13 Living dog's lungs. The bronchus of a division of lobules in one lobe obstructed with a valve, to allow only expiration for that part of the lungs, the action of the valve observed, the pulmonary vessels to the lobe ligated, the action of the valve observed again, chloroform given for inhalation by the remainder of the lungs, and the air from the obstructed division analyzed for chloroform.* A dog was anesthetized and tracheotomized. The dilatable cannula was introduced through the tracheotomy opening and fixed in the bronchial tree of the right lower lobe, at a point just distal to the origin of the first branch of the lobe. The cannula was connected to the submerged tube of the water-valve. With each expiration air passed through the valve and with each inspiration water rose into the submerged tube. The action was observed long enough to ascertain that it was continuous. Preparation was then made to open the chest. Without disturbing the cannula, positive pressure intratracheal insufflation of air and ether-vapor was given to maintain pulmonary aeration. Incision was made through the right 5th intercostal space, the chest was opened, and the hilus of the right lower lobe was exposed. The pulmonary artery and vein supplying that lobe were ligated securely. The chest was closed by suturing the wound care being exercised to inflate the lungs sufficiently and thus to avoid residual pneumothorax. The positive pressure insufflation was then discontinued. It was found that the dog breathed as before and that the action of the air at the water-valve was unaltered. A series of five small bottles, each half full of water, was prepared. The rubber tube connecting the cannula to the water-valve was disconnected at the water-valve, and its end was submerged

in the water in one of the bottles. When the air had bubbled through the water for one minute the tube was transferred to a second bottle and at the same time a few drops of chloroform were placed in the path of the respired air of the non cannulated parts of the lungs. After one minute the tube was taken to a third bottle and allowed to remain there for one minute. The two remaining bottles were used in succession in the same way. The water in the bottles was then tested for the presence of chloroform by the method of Ross (51). The first specimen was negative and the rest gave strongly positive reactions.

#### Deductions

11 Openings smaller in calibre than the terminal respiratory ducts and alveoli connect the arborizations of adjacent pulmonary lobules in the same lobe. This is deduced from the fact that India ink granules may pass collaterally from one lobule to another, while the larger bismuth crystals, which are readily injected through the bronchi to the alveoli do not pass collaterally. These openings are patent only when the alveoli are expanded to some degree beyond that of the state of collapse produced by removal of the lungs from the body, since India ink easily reaches the alveoli when the lungs are collapsed but passes collaterally from lobule to lobule only with expansion of the lungs.

12 The pulmonary circulation plays no essential part in the mechanism of collateral respiration.

13 Chloroform vapor passes readily with the air during collateral respiration.

#### Section 6

*Object To determine the degree of interference from the presence of fluids in the bronchi with the transfer of air collaterally between pulmonary lobules.*

##### Experiments *in vitro* and *in vivo* in the dog

*Protocol 14 Dog's lungs—one edematous lobe. The lobe expanded in a negative pressure chamber the bronchus of one lobule injected with air and the force of inflation necessary to transfer air collaterally between lobules measured, water injected into the same bronchus air again injected and the force of inflation necessary to transfer air collaterally between lobules measured. The lungs were obtained from a dog which had died from an overdose of sodium amyta. They were voluminous and heavy and the air passages were filled with frothy fluid of pulmonary edema. The right lower lobe was detached and prepared by fixing one cannula in the stem bronchus and another in the first*

branch. The specimen was then expanded by placing it in a negative pressure chamber at -12 cm H<sub>2</sub>O pressure. The cannula of the bronchial branch was connected with apparatus and injected with air, while the other was submerged in water and watched for escape of air. Escape was initiated at 20 cm H<sub>2</sub>O pressure. Now, 5 cc of water was injected into the lobe through the cannula used for injection of air, and the test was repeated. The same "initiation" reading was obtained. Ten cc of water was added, and another test gave an "initiation" value of 18 cm H<sub>2</sub>O.

*Protocol 15 Living dog's lungs. The bronchus of a division of lobules in one lobe obstructed with a valve, to allow only inspiration for that part of the lungs, the action of the valve observed, a colloidal solution (argyrol) injected into the cannula, the action of the valve observed again.* A dog was anesthetized and tracheotomized. The dilatable cannula was introduced and fixed in the bronchial tree of the right lower lobe, at a point just distal to the origin of the first branch. The cannula was connected to the elevated tube of the water-valve, Figure 3, B. With each inspiration air bubbled past the valve (into the cannula) and with expiration water rose into the tube a few centimeters. The action was observed long enough to ascertain that it was continuous, and then 5 cc of 10 per cent argyrol was injected into the lobe through the dilatable cannula. The action of the valve was observed again. No air passed it for the first 3 or 4 respirations, and after that the behavior was as before. Fifteen cc of argyrol was added, and this time the valve showed no passage of air at all. The water merely rose and fell in the tube.

#### *Deductions*

14 Collateral transfer of air between pulmonary lobules is entirely prevented by the presence of large amounts of water or a colloidal fluid in the air passages. Smaller amounts interfere less and may only slightly raise the resistance, and the intrabronchial fluid in marked pulmonary edema may not cause any appreciable resistance. The smaller amounts of fluid probably become so distributed and divided among the peripheral airways by the current of air as to leave parts free for collateral transfer.

#### DISCUSSION

Two possible sources of error were kept particularly in mind during the performance of these experiments, namely, leakage of air at the point of attachment of the dilatable cannula to the bronchial wall, and rupture of minute airways in the lungs. These appear to have been eliminated satisfactorily. First, leakage seemed highly improbable to all observers of the action of the dilatable cannula and of the

rigorous tests to which its attachment was submitted but still more convincing are the facts that the appearances of collateral transfer always failed to occur with the cannula fixed in the primary bronchi, and that in experiments *in vitro*, with the cannulas tied in the bronchi collateral transfer was obtained as well. Second, the extremely low pressures of injection used in producing the transfer artificially and the fact that the transfer is initiated and maintained spontaneously *in vivo* during quiet breathing appear to remove the possibility of alveolar rupture as an essential part of the process.

Collateral transfer of air in the lungs requires intimate fusion of the lobules and it is prevented by the presence of complete septa between the lobules. These circumstances have been found to vary considerably with different species and with different regions of the lungs. In man, as also, in the dog and cat, the interlobular septa are thin and incomplete while the interlobar septa are thick and complete. In these species air was found to pass freely from lobule to lobule only when they lay within the same lobe. Tests were applied to the major lobules alone, but it is likely that collateral transfer is possible also between minor lobules, considering the similarity of the two as to degree of fusion. Technical limitations prevented as extensive examination for collateral respiration in living animals as was applied for the transfer of air in specimens removed from the body, but it appears very likely that collateral respiration occurs in all parts of the lungs where collateral transfer of air was found possible.

The means by which air passes collaterally from lobule to lobule remains in doubt. Some light is thrown upon this by the behavior of gases, liquids and particulate matter injected into the air passages of a lobule. We conclude that anatomical openings must exist between the lobules at their planes of fusion that the openings lie between the terminal airways perhaps between the alveoli that they are extremely minute in caliber smaller than the alveoli and that they are patent only when the parenchyma is expanded. Of the structures of the lung with which we are acquainted, the pores of Kohn meet these criteria best. If these pores are normal structures they may well account for all of the appearances of collateral transfer. We are inclined to believe that collateral transfer of air depends also to some extent upon diffusion.

From the evidence at hand as to the minuteness of the openings between the lobules, one readily understands the reason for the negative results of the anatomical investigations of many workers as to the question of interlobular anastomosis. Whatever the openings may be, it is clear that they permit only very limpid fluids to pass. The masses which investigators have injected in making corrosion preparations have been far too viscid. Penetration of the injected material to the alveoli cannot be taken as adequate for the demonstration of interlobular connections—witness our negative results with bismuth. Moreover, the practice has been to inject the whole specimen at once from the stem bronchus, rather than to inject the bronchus of one lobule at a time, and this afforded poor opportunity for the masses to flow from lobule to lobule. Also, wax models of the lungs cannot be expected to demonstrate the presence of such minute structures as are the openings in question, for microsections cannot be cut thin enough.

The term, collateral respiration, is applied to the function described in this paper, because of the analogy which it bears to collateral circulation by capillary anastomosis in the blood-vascular system. The two functions are fairly similar in mechanism of operation and in physiological purpose.

Collateral respiration, like collateral circulation, depends for its occurrence upon the development of differences of pressure in two adjacent units of passages, and this takes place only when the main channel of supply for one unit is obstructed. The differences of pressure in the bronchial tree, between obstructed and free parts, have been measured directly in these experiments and were found to vary in magnitude with the depth of breathing. Only very slight differences, such as occur in light breathing, are required to carry on collateral respiration.

The physiological purpose of collateral respiration becomes apparent in reviewing the work in bronchial obstruction which has already been reported by members of our group. Collateral respiration has much the same economic rôle in the operation of the bronchial tree as collateral circulation has in that of the vascular system, for it acts to conserve the function of parts which have become obstructed. The small bronchial branches are obstructed much more frequently than are the blood vessels, in all probability, since occluding substances, especially products of inflammation, find very ready access to the air

passages Furthermore, the ebb and flow principle of aeration of the lungs is at a greater mechanical disadvantage in the presence of obstruction by fluids than is circulation Thus it is a matter of common observation in clinical fluoroscopy of the lungs with lipiodol in the bronchi for diagnostic purposes, that a column of lipiodol in a small bronchus remains there during respiration, rides to and fro, and permits no air to pass Its expulsion occurs only with forceful expiratory effort (cough) No more than a droplet of fluid is required to obstruct a capillary bronchiole in this way and, if it occurs from bronchial secretion at night or during any period when cough is absent for some time the obstruction must remain and the imprisoned air undergo absorption It is here that collateral respiration probably functions to maintain the air supply, until cough or another eliminative force may act Experiments with dogs by Lindskog and Van Allen (52) have shown that air may be absorbed from an obstructed lung lobe (incapable of collateral respiration) in sufficient quantity within 30 minutes to render cough entirely ineffectual for elimination Under these circumstances too the lobe becomes atelectatic as a rule within 24 hours Van Allen and Adams (53) and Van Allen and Lindskog (49 50) have found that the bronchus of one lobular division in a dog's lungs may be obstructed and remain fully air-containing for indefinitely long periods of time Adams (54) has repeated and subsequently confirmed this finding The lungs of man with chronic destructive diseases are being searched at autopsy for the presence of bronchiolar stenosis by Van Allen and Chin In the first specimen examined there was found occlusion of a tertiary bronchus at one point by a tuberculous lesion with the lobule which was supplied by the bronchus air containing Our experience both in man and in laboratory animals has been that lobular atelectasis develops after obstruction when in addition to occlusion of the bronchus of the lobule, there is occlusion of the airways at the periphery of the lobule upon which collateral respiration depends

#### SUMMARY

The air tightness of the pulmonary lobule has been investigated in several species of animal including man in order to determine whether air may pass collaterally from lobule to lobule This is found to be the

case in those species and in those parts of the lungs where the lobules are intimately fused and lack complete interlobular septa. In man collateral transfer of air between lobules is possible where the lobules belong to the same lobe, and not where they belong to different lobes. The transfer is very free. Thin liquids and finely particulate matter are also able to pass. The mode of transfer of air has not been surely determined, but it is supposed to depend both upon diffusion through the alveolar walls separating the lobules and upon flow through minute openings in these membranes.

After obstruction of the bronchus of one lobular division of a lobe in the lungs of living dogs, air is found to enter and to leave that part during the breathing by means of collateral connections with adjacent unobstructed lobules. To this function of the obstructed pulmonary lobule the term collateral respiration is given. It fails to occur between lobules situated in different lobes.

The physiological significance of collateral respiration is discussed in its relation to bronchial obstruction and pulmonary atelectasis.

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Further Studies in Obstructive Pulmonary Atelectasis

# THE HEALING OF RICKETS COINCIDENT WITH LOW SERUM INORGANIC PHOSPHORUS

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Within the past year, roentgenological evidence of healing of rickets coincident with a persistently low level of serum inorganic phosphorus has been observed in 2 children. These findings are of importance in relation to the theories of bone formation

The subjects of the study were 2 girls of 12 and 13 years of age respectively, both of whom showed roentgenological, clinical and chemical evidence of active rickets. The serum calcium values of both children were within normal limits the serum inorganic phosphorus markedly depressed. The children were given dietary treatment and kept under observation for a period of several months. During this time roentgenological evidence of marked healing of the bone was observed but the serum inorganic phosphorus of each child remained essentially at the same low level

## METHODS

Blood was drawn before breakfast and the serum separated from the clot as rapidly as possible. Calcium was determined by the method of Kramer and Tisdall (1) allowing the serum to stand 24 hours after the addition of the oxalate. The method of Fiske and Subbarow (2) was used for inorganic phosphorus protein was determined by micro Kjeldahl (3). All possible precautions were taken in order to insure accuracy in the determinations

## RESULTS AND DISCUSSION

Blood and metabolic studies of the 2 children were made at intervals during a period of several months. The values obtained for serum

calcium and phosphorus are given in table I. Reproductions of the roentgenograms of the bones are shown in figures 1 and 2. The films, as interpreted by representatives of the departments of roentgenology, orthopedics, and pediatrics, show marked evidence of progressive healing.

TABLE I

*The serum calcium and inorganic phosphorus of two children during healing rickets*

D C 13 years				M N 12 years			
Date	Cal cium mgm per cent	Phos phorus mgm per cent	Cal cium X phos phorus	Date	Cal cium mgm per cent	Phos phorus mgm per cent	Cal cium X phos phorus
July 17, 1929	9.9	3.2	31.7	January 3, 1930	11.3	2.2	24.9
July 26, 1929	11.4	3.0	34.2	February 12, 1930	10.2	2.2	22.4
October 11, 1929	10.6	3.0	31.8	February 18, 1930	10.4	2.4	25.0
October 18, 1929	10.3	3.1	31.9	June 17, 1930	10.8	2.0	21.6
October 29, 1929	10.8	2.5	27.0	June 24, 1930	11.1	2.2*	24.4
November 5, 1929	10.9	2.9	31.6				
November 17, 1929	10.5	2.8	29.4	August 5, 1930	11.2	3.2	35.8
February 18, 1930	9.6	2.4	23.0	November 11, 1930	10.9	2.7†	29.4
March 4, 1930	10.6	2.8	29.7				
March 25, 1930	10.2	2.7	27.5				
April 1, 1930	9.9	2.6	25.7				
April 9, 1930	10.3	2.8	28.8				
April 14, 1930	10.9	3.1	33.8				

\* Phosphatase 0.42 units (normal 0.12-0.21)

† Total acid soluble phosphorus, whole blood, 21.8 mgm per cent

Detailed reports of the clinical findings and studies of the mineral metabolism of the 2 children are reported elsewhere (4). The calcium and phosphorus findings, however, may be summarized briefly here. The child D C, in November, 1929, after more than 3 months of antirachitic treatment, was found to be retaining 25 mgm of calcium per kilo daily, with a high intake of this element. The daily retention

of phosphorus was still better, 30 mgm per kilo. In March, 1930, after the continuation of the same treatment, the daily retentions of calcium and phosphorus were 30 and 21 mgm per kilo respectively. The addition of 60 drops of viosterol<sup>1</sup> daily increased these retentions



FIG. 1 ROENTGENOGRAMS OF THE WRIST OF D.C.

*a* was taken July 24, 1929. *b* was taken October 4, 1929. *c* was taken March 31, 1930. During this time the child received antirachitic treatment. Metabolic observations indicated adequate retention of calcium and phosphorus yet the serum inorganic phosphorus was consistently low (2.5 to 3 mgm per 100 cc.) throughout the entire period.

to 39 and 29 mgm per kilo respectively. The child M.N. had been receiving antirachitic treatment for about 5 weeks previous to the first study. The daily retentions of calcium and phosphorus first observed were 24 and 15 mgm per kilo. Four months later, with no change in diet but with sunlight treatment in addition to the cod liver oil for one month previous to the observation period the daily calcium retention had increased to 30 mgm per kilo, the phosphorus retention remaining at the same level as in the previous study. The retentions of calcium and phosphorus observed in both children were thus well above the average normal retention as given by Sherman (5) and may be considered adequate for the healing of rickets. Serum analyses for calcium and inorganic phosphorus were made at intervals during the periods of study and are reported in table I. Of the other blood analyses, the values obtained for carbon dioxide capacity, for non protein nitrogen and for chloride were always within normal limits. Determinations of pH were not made.

<sup>1</sup> The viosterol used in these studies was supplied by Mead Johnson and Company.

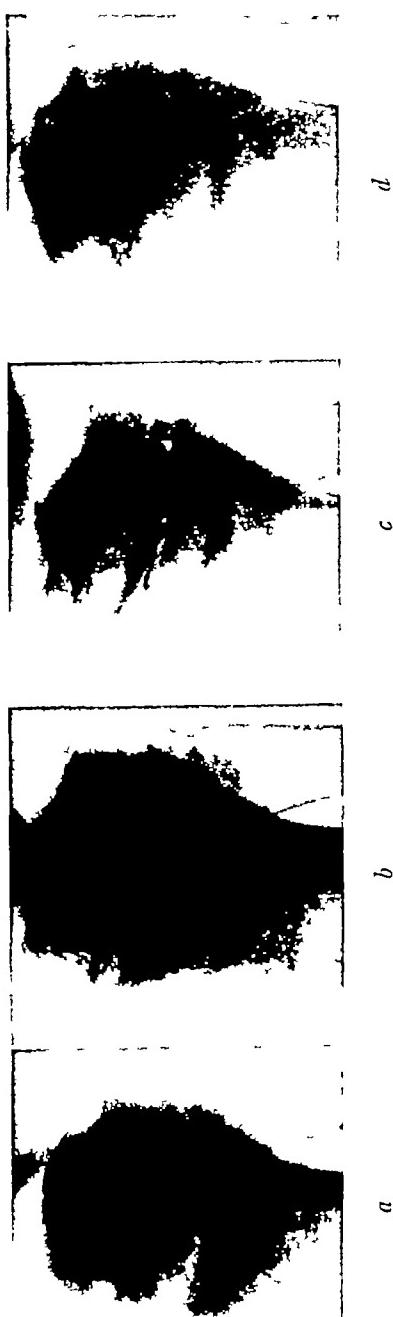


FIG. 2 ROENTGENOGRAMS OF PROXIMAL END OF TIBIA OF M N  
a was taken September 23, 1929, b was taken February 17, 1930, c was taken August 5, 1930, and d, November 13, 1930. During this time the child received antirachitic treatment. Metabolic observations indicated adequate retention of calcium and phosphorus, yet the serum inorganic phosphorus was consistently low (2 to 3.2 mgm per 100 cc) throughout the entire period.

The daily retentions of calcium and phosphorus observed in each child during these studies were amply sufficient to provide the minerals necessary for calcification of bone. It is evident from the roentgenograms that such calcification took place. Nevertheless the serum inorganic phosphorus of each child remained approximately at the same level, fluctuating between 2 and 3 mgm per cent. The existence of such values in cases of healing rickets is noteworthy because it has been generally accepted heretofore that serum phosphorus values as low as these are not compatible with healing rickets, but are indicative of rachitic activity. Almost as striking is the fact that in the child D C the rickets was active during the late summer and that despite the low serum inorganic phosphorus, the period of marked healing occurred during the late winter—a reversal of the usual seasonal activity and healing of rickets.

A careful review of the literature has disclosed but two other reports of healing rickets concomitant with low serum inorganic phosphorus, and in one of these reports the low phosphorus was but a transitory finding. Howland and Kramer (6) in their original series of observations of serum calcium and inorganic phosphorus in infantile rickets listed 2 children with "unmistakable evidence of healing" when the serum inorganic phosphorus values were 3.2 mgm per cent. The authors made no special comment on these cases but concluded that a serum inorganic phosphorus of 3 mgm per cent or less was indicative of active rickets. Gargill and his collaborators (7) have recently reported a case of osteomalacia, or adult rickets in a 38 year old woman, wherein definite increase in density of bone was observed during a period of ten months. The serum inorganic phosphorus of the patient remained consistently low during the entire study varying from 2.16 to 2.64 mgm per cent. Though this fact occasioned no comment by the authors we believe it to be of significance.

On the other hand, Hess Weinstock, Rivkin and Gross (8) have observed active rickets in infants whose serum calcium and inorganic phosphorus levels were quite normal. By proper manipulation of the diet, these authors were able to increase the serum inorganic phosphorus of rachitic rats to normal values, and yet delay markedly the healing of the rachitic lesions. These results were interpreted as indicative of the fact that both local and systemic factors were involved in the production of rickets.

The occurrence of calcification of bone despite a low serum inorganic phosphorus is most readily explained by considering that the deposit might consist almost wholly of calcium carbonate, instead of the normal calcium phosphate-carbonate complex. This explanation, however, is not satisfactory in the cases studied by us, in view of the large amounts of phosphorus retained by each child. The amount of phosphorus utilized in the production of tissues other than bone may be estimated roughly from the retention of nitrogen, the ratio of N:P in the soft tissues being approximately 17:1 (9). The maximum retention of nitrogen observed in either child, 87 mgm per kilo, can account for only about 5 mgm per kilo of the retained phosphorus. A daily storage of from 10 to 24 mgm of phosphorus per kilo body weight in tissues other than bone seems even more difficult to reconcile with the maintenance of the low serum inorganic phosphorus than does its storage in bone.

If it may be granted that at least a part of the deposit must consist of calcium phosphate, the occurrence of this marked deposition of minerals at the metaphyses, coincident with the maintenance of a low serum inorganic phosphorus, must have a direct bearing upon the theories of calcification of bone. Any theory, to be adequate, must explain the calcification which occurs under unusual conditions such as those described here.

The modern theories of normal calcification of bone may be divided into 2 groups: those concerned primarily with conditions existing in serum, as related to the probable precipitation of insoluble calcium salts in bone, and those concerned only with conditions at the site of bone formation. In the first group, Shear and Kramer (10) have brought forward evidence to prove that the calcium phosphate compound of blood serum is the dicalcium, instead of the tricalcium salt. According to these authors, the serum of children is normally saturated, or nearly saturated, with dicalcium phosphate. Calcification of bone was obtained *in vitro* only when the bone was immersed in solutions containing sufficient calcium and phosphate ions so that the ion product  $(Ca^{''}) \times (HPO_4^{-})$  was greater than  $2.5 \times 10^{-6}$  an ion product corresponding to an empirical product of calcium  $\times$  phosphorus = 35. In serum, the calcium is not wholly ionized, therefore the empirical product of serum calcium and phosphorus represents a

greater value than the ion product  $(\text{Ca}^{++}) \times (\text{HPO}_4^{2-})$ . Because calcification did not take place *in vitro* when the empirical product calcium  $\times$  phosphorus was less than 35, these authors postulated that calcification *in vivo* would not occur until the empirical product reached a value of 35 or greater.

An examination of the empirical products calcium  $\times$  phosphorus of the serum of the child D C (table I) reveals but 2 values approximating 35 and one of these values was obtained when the rachitic process was most active. A product of 36 was obtained with the second child M N, during the period of greatest healing. Other values for this product obtained during healing were as low as 22. According to the calculations of Shear and Kramer, the serums of both children were undersaturated with dicalcium phosphate to a degree that would preclude calcification entirely. Yet calcification occurred in both cases, as is shown in figures 1 and 2.

In 1925, Holt, La Mer and Chown (11) studied the serum calcium and inorganic phosphorus relative to the solubility product of tricalcium phosphate, concluding that normal serum is supersaturated with tricalcium phosphate to the extent of about 200 per cent, and that even in active rickets the serum is still supersaturated with tricalcium phosphate. Holt (12) suggested that calcification is not entirely arrested in active rickets, but that it is so retarded that bone growth exceeds it in activity, thus producing the inadequately calcified bone characteristic of rickets.

It might be argued, from this theory, that in these cases of late rickets, growth of bone was slow enough so that even though calcification was much decreased, it was still carried on sufficiently to make a noticeable difference in the density of the bone during the period studied. Such an explanation is unsatisfactory in many respects. The first roentgenograms are typical of active rickets. The child D C grew an inch in height during the 9 months observation period, so the growth of bone was not inordinately slow. The retentions of calcium and phosphorus observed in each child were of the order of healing rickets—the calcification which occurred was apparently of the type characteristic of healing, not of active rickets. In every respect excepting the low serum inorganic phosphorus, the picture resembles the healing observed in infantile rickets when the serum phosphorus

tration of phosphate ion *at the point of calcification* could be increased to such an extent that the solubility product of calcium phosphate would be exceeded and precipitation take place. These authors also demonstrated that in rickets of rats the amount of bone phosphatase is not decreased but is probably increased. The failure of calcification in rickets, therefore, is probably not due to lack of enzyme, but to some other factor, as a deficient amount of substrate.

Phosphate esters which can be hydrolyzed by the bone phosphatase are found in the red corpuscles, leucocytes, and to a slight extent in the blood plasma (18). The amounts of these esters in other body fluids are not known. Small amounts of phosphatase having the same properties as bone phosphatase and presumably identical with it are found in plasma (19). Kay (20) has recently published data showing that in certain diseases of man wherein there is destruction of bone, or lack of deposition of bone salts, the phosphatase content of plasma is markedly increased. In 10 cases of infantile rickets, reported by him, the plasma phosphatase averaged 0.95 units (the normal for this age group is 0.17-0.34 units), in one case of adolescent rickets the very high value of 2.4 units was observed. The plasma phosphatase decreases when the child is given antirachitic dietary treatment (21). A study of the phosphatase content of the blood of our patients seemed desirable. Through the courtesy of Dr. Kay, an analysis of the phosphatase content of the plasma of M. N. was obtained. The child had been receiving antirachitic treatment for about 6 months and evidences of healing were very definite. The value found, 0.42 units, is definitely above normal (0.12-0.20 units, normal adult range) but far below the values noted in Kay's cases of untreated rickets. If the amount of plasma phosphatase is indicative of the amount available in bone, there should be sufficient enzyme present for normal calcification.

In order to account for the calcification observed in these 2 children with late rickets according to Robison's theory, it is necessary to postulate the hydrolysis of an unusually large amount of phosphate ester to provide sufficient phosphate ion at the metaphyses for the precipitation of calcium phosphate. Such an increased hydrolysis is within the realms of possibility, although the data obtained are insufficient either to support or to deny such a conclusion. A single

determination of total acid soluble phosphorus of whole blood of M N indicated that the total ester phosphorus was within normal limits. Whether the fraction of phosphate ester which can be hydrolyzed by bone enzyme is normal or below normal has not been determined. It is hoped that these studies may be continued.

#### SUMMARY

The clinical healing of late rickets coincident with a persistently low level of serum inorganic phosphorus has been observed in 2 children.

The retention of calcium and phosphorus observed in each child was considered amply sufficient for the building of bone.

The data herein reported do not offer evidence definitely substantiating any of the prevalent theories of bone calcification, moreover, it is difficult to reconcile certain of these theories with the findings.

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# A QUASI-CONTINUOUS RECORDER FOR OXYGEN AND CARBON DIOXIDE FOR CLINICAL ATMOSPHERE CONTROL<sup>1</sup>

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Rapid and dependable methods of gas analysis are essential in the operation of tents and cubicles if specific concentrations of oxygen and carbon dioxide are to be maintained for treatment. From a clinical standpoint chemical methods of analysis have the following distinct limitations. Unless tests are performed at very frequent intervals a complete record of variations is not obtained. Individual determinations are time consuming, usually requiring from 5 to 10 minutes, while the apparatus and solutions need frequent attention and their correct use involves considerable technical skill.

Recent application of the thermal conductivity method of gas analysis for automatically recording oxygen and carbon dioxide in air has greatly simplified the problem of clinical atmosphere control in the atmosphere control room operated at Harlem Hospital. Oxygen losses due to leaks, or accompanying the opening of the door are promptly indicated on the recorder chart, and can thus be reduced to a minimum and compensated by increased flow while the chart is obviously helpful in controlling decarbonation. The daily record promotes economy in operation as well as the safety of the patients and by providing an accurate check on the conditions of operation it renders them less subject to human factors (1, 7).

Since thermal conductivity instruments calibrated for the direct measurement of oxygen in air, and also for the measurement of carbon dioxide in the presence of high and fluctuating oxygen tensions, have

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<sup>1</sup> Supported by the Committee for the Encouragement of Medical Research and the Littauer Pneumonia Research Fund of New York University

hitherto been unavailable, a brief account will be presented of the instrument, its development and the method of its operation.

#### THERMAL CONDUCTIVITY METHOD OF GAS ANALYSIS

Within the past few years considerable impetus has been given to the technical development and industrial application of gas analysis methods based on differences in specific thermal conductivity. Indicating and recording instruments have been devised which detect the presence of an impurity in a given gas, or of quantitative variations in the composition of a gas mixture by means of changes in the temperature of an electrically heated platinum filament, which is surrounded by a thin layer of the gas to be tested. The lower the thermal conductivity of this gas, the greater will be its thermal insulating capacity, and hence the higher the equilibrium temperature and the electrical resistance of the filament.

The changes in the electrical resistance of the filament are directly referred to those of an identical filament, which is heated by the same current, but is insulated by a standard or reference gas mixture, and is contained in a separate compartment of the thermal cell. For purposes of comparison, the two filaments form adjacent arms of a Wheatstone bridge, the other two arms of the bridge being thermally indifferent manganin coils of approximately equal resistance, usually adjustable by means of a slide wire. If the bridge is balanced when the two compartments contain the same gas mixture (by adjustment of the slide wire or by some mechanical means of controlling the rate of heat loss from the platinum filaments) any difference between the composition of the mixture to be analyzed and the reference mixture will disturb the electrical balance of the bridge to an extent dependent on the difference between the thermal conductivities of the component gases as well as on the quantitative change in their relative proportions.

The thermal conductivity of the mixture is not always a linear function of the relative concentrations of the components. For water vapor in air, for example, the relation is far from linear, showing a distinct maximum at about 20 per cent water vapor (2). For carbon dioxide in air the relation appears to be linear at least up to 10 per cent carbon dioxide. For oxygen and nitrogen mixtures there are some apparent

departures from a linear relation, possibly due to variations in the concentration of contaminating gases such as argon or hydrogen (3).

In the case of the Engelhard thermal cell, which was used in the present studies, the platinum filament is spiral in form and is fused into a quartz tube. It is claimed that the rigid support of the quartz insures a constant electrical resistance for a given temperature by eliminating variable factors due to the thermal expansion and contraction of the bare wire, with attendant changes in mechanical strain (3). To prevent the cooling action of a rapid flow of gas, only a small portion of the gas stream is passed through narrow openings into the thermal compartment proper.

The Engelhard cell has two devices for adjusting the cell readings to zero irrespective of whether the two compartments contain identical or different gas mixtures. One method consists in mechanically altering the rate of heat loss from one of the platinum filaments by a movable brass sleeve, referred to as the compensating plug. The sleeve can be raised or lowered by an adjusting screw, which is situated at the base of the compartment and is protected from air leakage by a screw cap. Raising the sleeve increases and lowering it decreases the rate of heat dissipation. The second method of balancing the Wheatstone bridge consists in changing the ratio of the balancing resistance wires by means of a slide wire screw situated at the top of the cell.

A description of the Engelhard instrument, with emphasis on the present modification for oxygen and carbon dioxide is given by Hamilton (3). Other types and makes of thermal conductivity cells are described by Knipping (5), Rabinowitch and Bazin (9), Palmer and Weaver (8) and Hill (4).

This physical method of analysis is not qualitative, serving merely to indicate quantitative changes in the proportions of the known ingredients of a gaseous mixture. Every instrument must be empirically calibrated with each type of mixture for which it is to be used.

In testing for variations of a component that is the only independent variable of a gas mixture, a single bypass thermal cell may be used. In this type of cell the gas stream is drawn past only one of the two compartments of the cell, the 'analysis' compartment while a mixture of fixed composition is sealed in the other "reference" compartment.

When the component to be measured is not the only independent variable, and the disturbing gases cannot be eliminated from the stream with suitable absorbents, a double by-pass cell must be used. In this type of cell the gas stream is by-passed through both compartments of the cell, but a scrubber is inserted before the reference compartment to remove completely the component, the concentration of which is to be measured.

#### MEASUREMENT OF CARBON DIOXIDE AND OXYGEN IN AIR MIXTURE

For the postulated reason that oxygen and nitrogen or air have closely similar thermal conductivities, and are thus not to be differentiated readily by the thermal conductivity method, suitably calibrated instruments have unfortunately not been hitherto available for the estimation of oxygen in air mixtures. The impression that oxygen and nitrogen are thermally indistinguishable appears to have been based upon the recorded thermal conductivity values at a temperature of 0° C., which is considerably lower than the temperature actually obtaining in the thermal cells (about 75 or 80° C.).

In Table 1 are presented Eucken's values for the thermal conductivi-

TABLE 1  
*Thermal conductivities of gases*

Gas	$k \times 10^6$		$(k_{\text{air}} - k_{\text{gas}}) \times 10^6$	
	0° C.	100° C.	0° C.	100° C.
Air	56.6	71.9		
Argon	38.8	50.9	17.8	21.0
Carbon dioxide	33.2	49.6	23.4	22.3
Nitrogen	56.8	71.8	-0.2	0.1
Oxygen	57.0	74.3	-0.4	-2.4

Sensitivity ratio  $\frac{O_2 \text{ in air}}{CO_2 \text{ in air}} = \frac{k_{\text{air}} - k_{O_2}}{k_{\text{air}} - k_{CO_2}} = -0.017 \text{ at } 0^\circ \text{ C} \text{ and } -0.108 \text{ at } 100^\circ \text{ C}$

$k$  is the amount of heat in gram calories flowing per second through an area of 1 sq cm with a temperature gradient of 1° C per cm. It increases with the temperature but is almost independent of pressure. The values of  $k$  are reproduced from Palmer and Weaver (8) and refer to experiments by Eucken.

TABLE 2  
*Calibration tests for recorder*

Volumes per cent oxygen January 7-28 1931			Volumes per cent carbon dioxide April 7-8 1931		
Recorder cell no 28123	Chemical analysis	Correction for recorder	Recorder cell no 29378	Chemical analysis	Correction for recorder
volumes per cent	volumes per cent	volumes per cent	volumes per cent	volumes per cent	volumes per cent
0.05	Nitrogen	-0.05			
17.5	Room air	+3.3	0.01	0.00	-0.01
17.5	Room air	+3.3	0.78	0.70	-0.08
18.5	Room air	+2.3	0.78	0.70	-0.08
25.4	27.04	+1.64	0.87	0.89	+0.02
25.7	26.67	+0.97	0.88	0.90	+0.02
31.3	31.5	+0.20	1.09	1.11	+0.02
31.4	31.9	+0.50	1.11	1.10	-0.01
31.6	31.8	+0.20	1.18	1.10	-0.08
34.8	33.5	-1.30	1.22	1.25	+0.03
35.0	33.7	-1.3	1.23	1.29	+0.06
35.7	34.6	-1.1	2.52	2.51	-0.01
40.3	38.6	-1.7	2.53	2.54	+0.01
40.5	38.96	-1.54	5.03	5.10	+0.07
41.5	39.76	-1.74			
41.6	39.7	-1.9			
42.0	39.3	-2.7			
49.8	45.6	-4.2			
50.1	45.7	-4.4			
50.2	46.2	-4.0			
50.3	45.3	-5.0			
54.9	50.3	-4.6			
54.9	50.15	-4.75			
61.5	55.42	-6.08			

ties of air, oxygen, nitrogen and carbon dioxide at 0° and 100° C (8). It will be seen that the values for oxygen and air are almost indistinguishable at 0° but show a greater divergence at 100°. At the higher temperature the difference between oxygen and air is about one tenth of that obtaining between carbon dioxide and air. The ratio has a negative sign, because the thermal conductivity of oxygen is higher, while that of carbon dioxide is lower than the value for air.

Thermal conductivity instruments of the single by-pass type are available for the estimation of carbon dioxide in air to within about  $\pm 0.05$  parts per hundred among others, the Engelhard, the Brown and the Leeds and Northrup carbon dioxide recorders and the "ka

therometer" of the Cambridge Instrument Co. On the basis of the ratio quoted above, it is somewhat surprising that no attempt has been made to adapt these instruments as oxygen in air recorders, having an accuracy of about 0.5 per cent. A single exception, of theoretical rather than practical interest, may be mentioned. Ledig and Lyman (6) describe a thermal conductivity oxygen in air indicator, constructed for respiratory studies under the auspices of the Bureau of Standards. The results obtained with this instrument agreed with chemical analyses to within 0.05 per cent for both oxygen and carbon dioxide. Because of the extreme delicacy of the oxygen cell and bridge used in this case, the instrument was subject to many disturbing factors and could not be converted into a continuous recorder.

Not only has the thermal conductivity method been unavailable for oxygen in air measurements, but in addition, when the method was applied to carbon dioxide measurements, the disturbing effect of a varying oxygen-nitrogen ratio was in general neglected. According to the value of the ratio  $\left( \frac{k_{\text{air}} - k_{\text{O}_2}}{k_{\text{air}} - k_{\text{CO}_2}} \right)_{100^\circ \text{C}}$  shown in Table 1, a single by-pass carbon dioxide cell with ordinary air sealed in the reference compartment should show an error of about 1 per cent in the carbon dioxide reading for a 10 per cent change in oxygen concentration, increase in oxygen appearing as an apparent decrease in carbon dioxide, and *vice versa*. Neglect of this source of error is justified only by virtue of the usual constancy of the carbon dioxide-oxygen ratio in industrial and in metabolic conditions, when the instrument has been calibrated under the conditions for which it is to be used. In this connection it should be mentioned that although Ledig and Lyman followed the general custom of using a single by-pass cell for carbon dioxide in air measurements, they nevertheless recognized the disturbing effect of a varying oxygen-nitrogen ratio, and compensated for this source of error by special correction factors.

#### DEVELOPMENT OF OXYGEN RECORDER

The development of a practical recorder for carbon dioxide and oxygen in air occurred in the following way. In 1928 a carbon dioxide recorder with a scale adjusted to read the carbon dioxide directly, up to a maximum of ten per cent, was made available for the use of one of us through the courtesy of Charles Engelhard, Inc., of Newark, N.J. The instrument was

of the usual single bypass type and was intended for alveolar air studies on patients receiving oxygen-enriched air. When the apparatus proved unsuitable for this purpose owing to the time lag involved and also because of the then unsuspected effect of the oxygen, it was continued in use as a part of the equipment of the atmosphere control room for pneumonia patients at Harlem Hospital to provide a continuous record of the carbon dioxide content of the air.

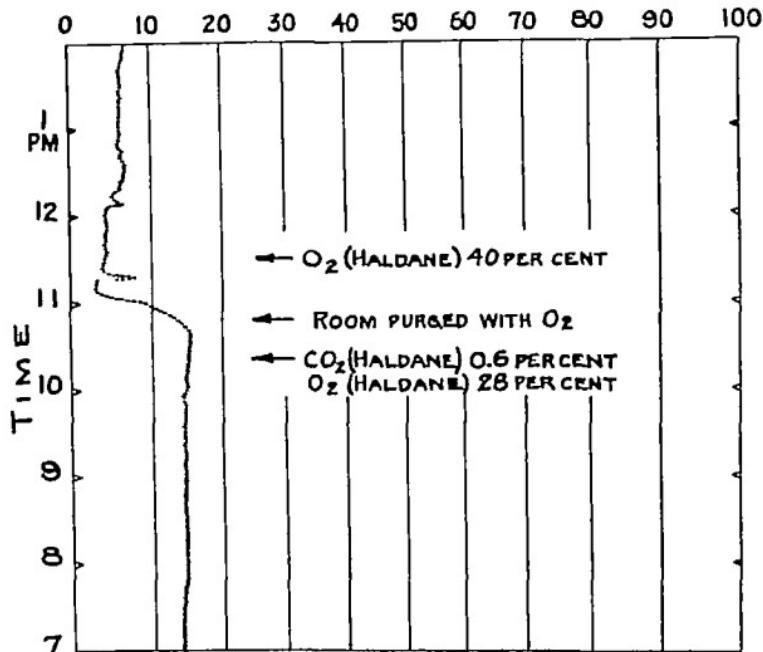


FIG. 1 INACCURATE CARBON DIOXIDE RECORD (PARTS PER MILLE) FROM OXYGEN CHAMBER WITH SINGLE BY PASS CELL

Here again the fluctuating oxygen concentration in the oxygen room rendered the instrument useless as a carbon dioxide recorder. Figure 1 shows a typical example of the inaccurate curves obtained in June 1929. It will be seen that the recorder shows 1.5 per cent carbon dioxide as opposed to 0.6 by chemical analysis. The oxygen concentration determined chemically at the same time was 28 per cent.

By means of the special compensating plug with which this particular type of instrument is equipped (see page 605) the cell had been adjusted so as to give zero deflection when the analysis compartment contained dry carbon dioxide free air drawn from the oxygen room and containing a high but undetermined oxygen content while the reference compartment con-

tained ordinary dried air, free from carbon dioxide. We can roughly estimate this undetermined oxygen concentration from the error in the recorded carbon dioxide. Assuming a sensitivity ratio of roughly 8 to 1 for carbon dioxide and oxygen respectively, as was indicated by subsequent tests with this instrument, the oxygen concentration at which the instrument had been originally balanced was probably about  $28 + 8(15 - 0.6) = 35.2$  per cent. Departures from this original oxygen concentration affected the accuracy of the carbon dioxide readings, a lower oxygen concentration giving too high a reading and a higher oxygen concentration having the opposite effect.

The above interpretation of the readings shown by the supposed carbon dioxide recorder was not made until December, 1929, when the anomalous results obtained were studied more closely. At that time Engelhard, Inc., readjusted the instrument by means of the compensating plug to give a zero deflection when the analysis compartment contained ordinary dry carbon dioxide-free air. Subsequent tests with dry room air (20-21 per cent oxygen) enriched with carbon dioxide showed results in agreement with Haldane determinations of the carbon dioxide. When the instrument was later connected with the dried oxygen-enriched air of the atmosphere control room, however, a negative deflection of the galvanometer was obtained, in spite of the presence of about 10 per cent of carbon dioxide. After testing for possible disturbing factors, such as leaks in the pipe lines, or insufficient dehydration of the air current, we notified the Engelhard laboratory of the apparently inaccurate results, for which they were unable to account.

It then occurred to us that the instrument might be more sensitive to variations in oxygen concentration than had been generally assumed to be the case. To test our supposition, it was necessary to observe the free deflection of the instrument in the presence of a varying oxygen concentration and under constant conditions of moisture and carbon dioxide. Since increase in oxygen apparently induced a negative deflection, and since the mechanical construction of the galvanometer prevented the index from swinging over more than two or three scale divisions below zero, the compensating plug of the cell was turned to unbalance the bridge, causing the recorder index to come to rest at about 65/100 of a full scale deflection with ordinary dry carbon dioxide-free air in both the analysis and the reference compartments of the cell. When a stream of dried carbon dioxide free air containing about 40 per cent oxygen was drawn through the analysis compartment from the oxygen room, the galvanometer index retreated to the left, as may be seen in Figure 2. The instrument appeared capable of registering a slight loss of oxygen when the nurse's lock to the oxygen room was opened, as well as the effect of increasing the rate of oxygen delivery to the room. When connected to an oxygen cylinder, a much more marked negative deflection was obtained. Calibration with a Binger absorption apparatus indicated that the sensitiveness toward oxygen was roughly one-

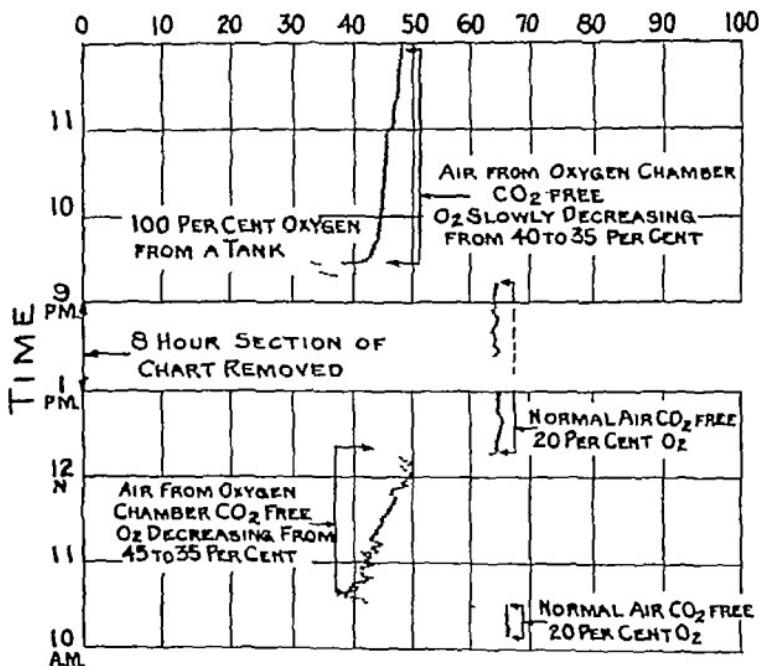


FIG. 2 EFFECT ON SINGLE BI PASS CELL OF VARIATIONS IN OXYGEN CONCENTRATION

eighth of that shown in the opposite direction toward carbon dioxide. It is of interest to note the close correlation between the sensitivity ratio as determined empirically  $\frac{O_2 \text{ in air}}{CO_2 \text{ in air}} = -1/8$  and the ratio calculated from the thermal conductivity values given in Table 1 for 100° C. namely  $\frac{O_2 \text{ in air}}{CO_2 \text{ in air}} = -24/223 = -1/9.3$

Since an accuracy of  $\pm 10$  or 20 per cent is quite adequate for the oxygen control in the clinical chamber the doubly practical significance of our observations became at once apparent. In the first place the single bi pass cell with galvanometer leads reversed appeared capable of functioning as an oxygen recorder for air freed from moisture and carbon dioxide. In the second place a cell of this type would be useless for carbon dioxide determinations of the air from the oxygen room. For this purpose a double bi pass cell should be used the standard compartment of which should receive dried carbon dioxide free air having the same oxygen nitrogen ratio as is present in the dry air drawn through the analysis compartment.

## RECORDER FOR OXYGEN AND CARBON DIOXIDE

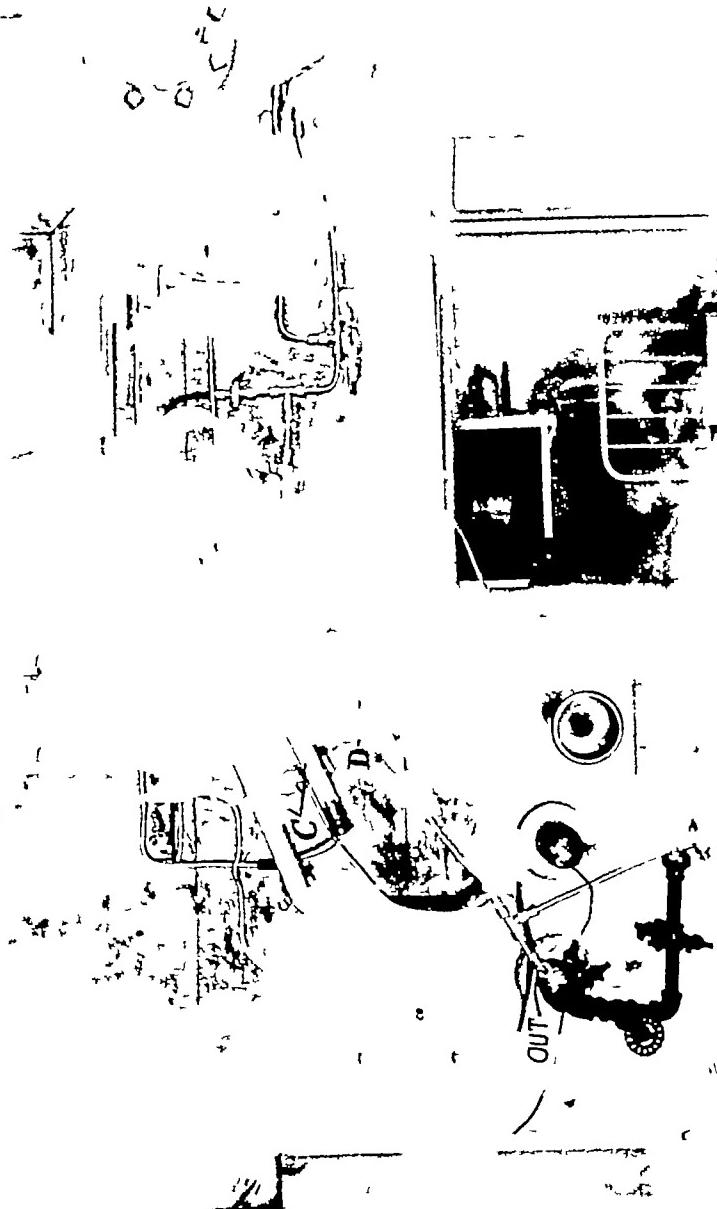


FIG. 3 CARBON DIOXIDE AND OXYGEN RECORDER FOR ATMOSPHERE CONTROL ROOM AT HARI L M HOSPITAL THERMAL CONDUCTIVITY CELLS AND ABSORPTION TRAIN  
A, double bypass carbon dioxide cell, showing J, the compensating plug, and K, the screw for adjusting the bridge slide wire. B, single bypass oxygen cell in air thermostat C, drying tubes, D, decarbonating tube, I, rheostat and milliammeter for carbon dioxide cell G, rheostat and milliammeter for oxygen cell tube, II, connection leading to rotary pump on the floor (not shown) I, connection leading from pump, OUT, II, connection leading to atmosphere control room IN, sample return duct  
the atmosphere control room can be connected to the "out" outlet.

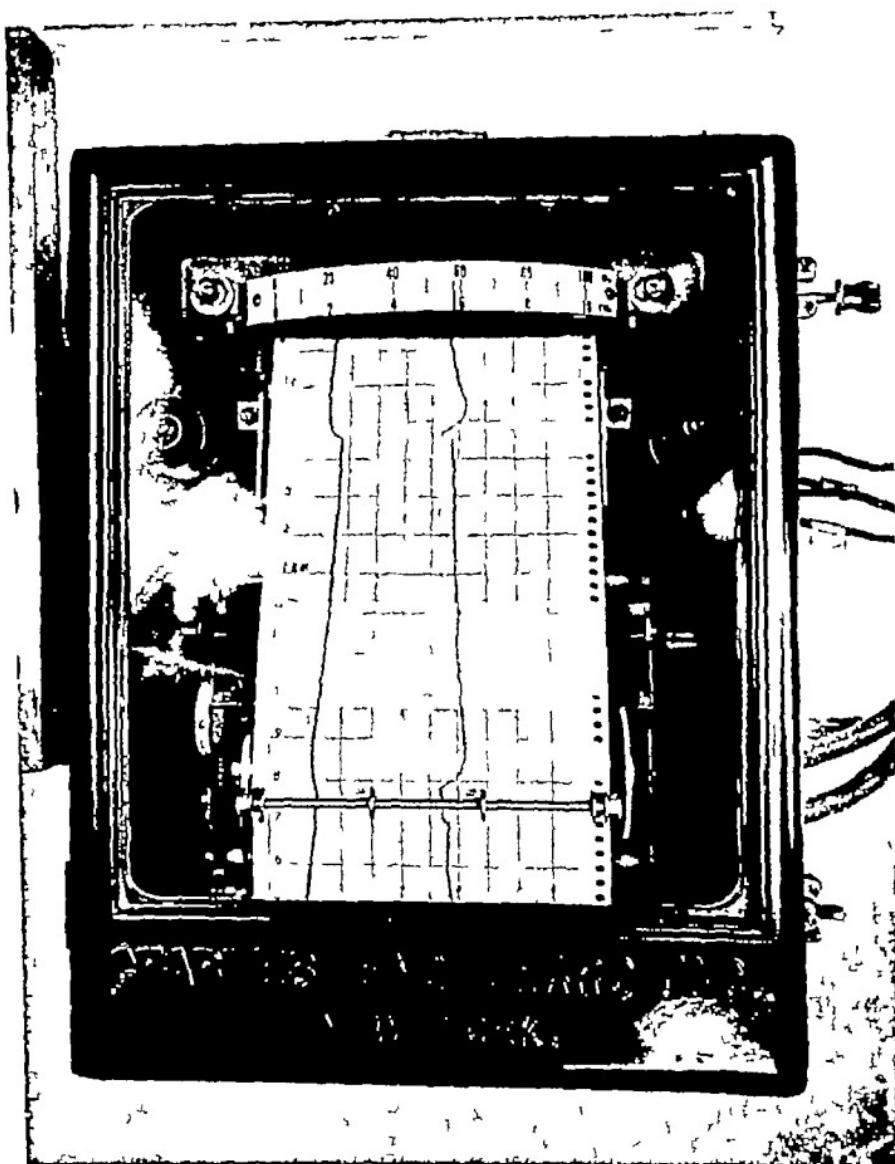


FIG 3a CARBON DIOXIDE AND OXYGEN RECORDER FOR ATMOSPHERE CONTROL ROOM AT HARLEM HOSPITAL RECORDING GALVANOMETER SHOWING 14 PER CENT CARBON DIOXIDE (HEAVY LINE) AND 58 PER CENT OXYGEN (LIGHTER LINE RED ON ORIGINAL CHART)

## THE CARBON DIOXIDE AND OXYGEN RECORDER

Charles Engelhard, Inc., promptly cooperated with us to the extent of constructing and calibrating a recording instrument for both carbon dioxide and oxygen, embodying the principle stated above. The instrument was installed for use at the hospital on February 21, 1930. The double and single by-pass cells, for carbon dioxide and oxygen respectively, and the absorption tubes are shown in Figure 3 attached to a vertical panel on the outer wall of the atmosphere control room. The cells are connected to an especially sensitive 2-point recording galvanometer with a 30-second alternating contact. The recorder (Fig. 3a) is attached to the opposite wall, where it is free from disturbing vibrations. The suction pump and batteries are not shown in the photographs.

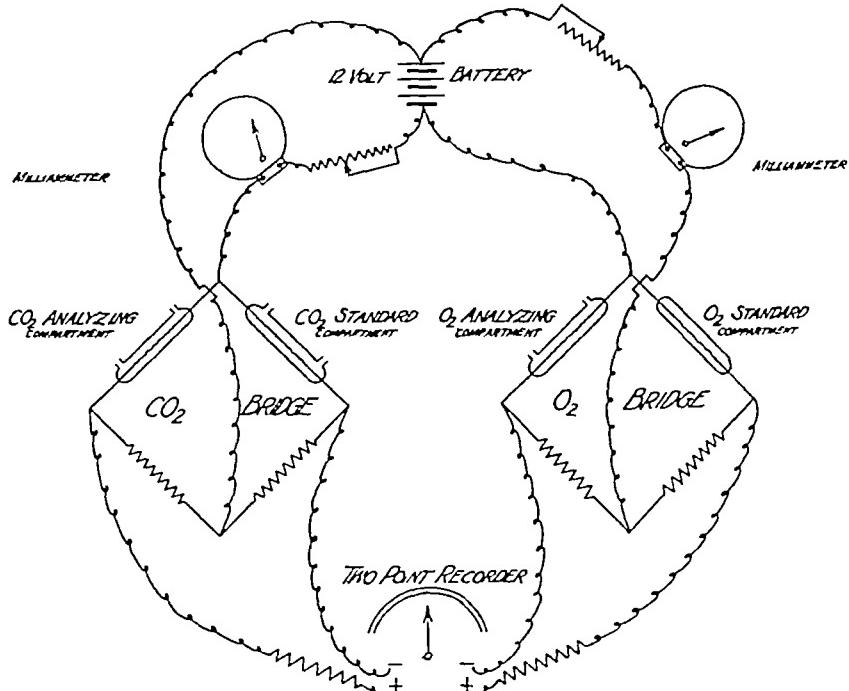


FIG. 4 CARBON DIOXIDE AND OXYGEN RECORDER ELECTRICAL DIAGRAM

It was found necessary to enclose the single by-pass cell in an air thermostat, which is maintained at 100° F by current from the 110-volt circuit. This precaution was not necessary in the case of the

double bypass cell which is less sensitive to fluctuations in room temperature. The current through the two bridges which is maintained at 240 milliamperes by separate milliammeters and rheostats is supplied by a 12 volt storage battery. The main electrical connections are shown in Figure 4. For charging purposes two batteries are kept in constant use, one being connected to the recorder while the other is on charge on the 110 D C house circuit, the voltage being diminished by 80 ohms series resistance. The change is made daily by the reversal of two double throw switches. The battery connections are shown in Figure 5. The current for the magnetic

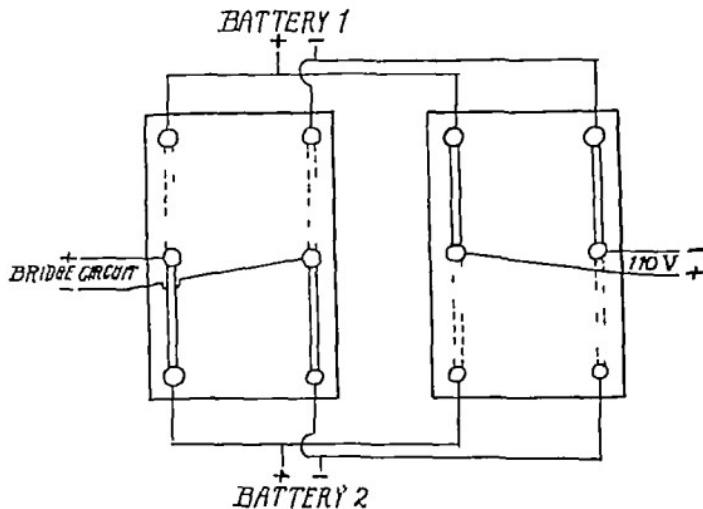


FIG 5 BATTERY CONNECTIONS

Two double throw switches used for reversing battery connections one battery being on charge while the other is being discharged through the bridge circuits of the thermal conductivity cells

alternating contact of the recorder is supplied through a reducing coil from the 110 volt circuit. The small rotary pump used to maintain a constant flow of gas through the cells, is not shown in the picture. It is lubricated by graphite or Aquadag, since oils are to be avoided in the presence of a high oxygen concentration.

*Absorbents* It has been found advisable to depart somewhat from the absorption arrangement described by Hamilton (3) in which sodium

hydroxide sticks were used as decarbonating agent and the air to be sampled was dried by calcium chloride before being split into the two streams for the double by-pass cell. In the first place, sodium hydroxide, both in stick and in flake form seemed poorly adapted for decarbonation of the air stream. The sticks offer insufficient reacting surface and rapidly become coated with carbonate, while, except in the presence of a high carbon dioxide and a low moisture content, the more efficient flakes tend to fuse into a solid mass which clogs the absorption tube.

To overcome this difficulty we substituted soda lime (15 per cent moisture, 4-6 mesh) as decarbonating agent, while retaining calcium chloride of the same mesh for dehydration. In the second place, since the reaction occurs with less efficiency from a thoroughly dried gas, we arranged for division of the gas samples into streams I and II before desiccation, but placed a drying tube after the decarbonating tube in the path of stream II. This arrangement provides for the complete absorption of the water of carbonation formed according to the equation,  $\text{CO}_2 + 2\text{NaOH} = \text{Na}_2\text{CO}_3 + \text{H}_2\text{O}$ .

Our present scheme is shown in Figure 6. Stream 1 refers to the dehydrated air stream which by-passes the analyzing compartment of the carbon dioxide cell. Stream 2 refers to the decarbonated and dehydrated air stream which by-passes the reference compartment of the carbon dioxide cell and the analyzing compartment of the oxygen cell.

Glass absorption tubes, measuring 18 inches in length and 1 inch in bore, and tapered to a tubular opening at one end, are used in preference to wide tubes or towers since they can be more rapidly replaced and cleaned, and the small bore of the single stopper makes them less liable to leak. The matter of ease in cleaning out the containers is of importance since continuous analysis involves frequent replacement of the absorbents. Thick-walled drying towers of the usual type are troublesome to clean, being readily cracked by the heat developed in dissolving out the partially caked calcium chloride. The drying tubes should be supported in a slanting position with the inlet uppermost, so that the moist calcium chloride flows slowly down the tube and cannot flow back out of the absorption tube to clog the narrow connecting tubes. Large U-tubes could conveniently be used. The absorption tubes should be

changed when the moisture has advanced one-quarter the length of the tube.

*Connections and flow regulation.* The connective tubing T-pieces and stopcocks, shown in Figure 6 are of copper ( $\frac{1}{4}$  inch) all the permanent joints being made with copper couplings coated with aluminum paint. To eliminate loss of oxygen the gas stream is returned from the pump to the chamber from which the sample was drawn. For ease in testing the recorder with room air or with commercial oxygen or in shifting the connections from one atmosphere control room to another, additional inlet and outlet tubes are provided through

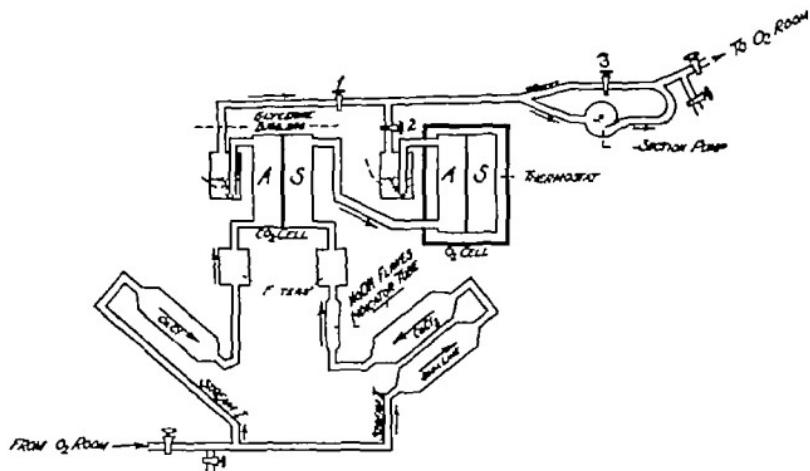


FIG 6 FLOW DIAGRAM OF CARBON DIOXIDE AND OXYGEN RECORDER

Analyzing compartment of cell S standard compartment of cell CO<sub>2</sub> cell O<sub>2</sub> + N<sub>2</sub> + CO in compartment A O<sub>2</sub> + N<sub>2</sub> in compartment S O<sub>2</sub> cell O<sub>2</sub> + N<sub>2</sub> in compartment A normal air mixture (dried) in compartment S

T piece connections and the connections to the oxygen chamber can be closed by means of two stopcocks

As may be seen from Figure 6, the rate of suction may be decreased from the maximum capacity of the pump by partially opening stopcock 3 in a shunt line connecting the inlet to the outlet of the pump. The rate of gas flow for the split streams is further and individually controlled by cocks 1 and 2 and is measured with the aid of glycerine

bubbler 1 and 2. The rate should be the same in the two streams and should be adjusted to between 80 and 120 bubbles per minute (about 250 cc per minute) since at this rate of flow neither the double nor the single by-pass cell is appreciably sensitive to slight variations in flow rate.

*Calibration.* In calibrating the instrument at the factory, the

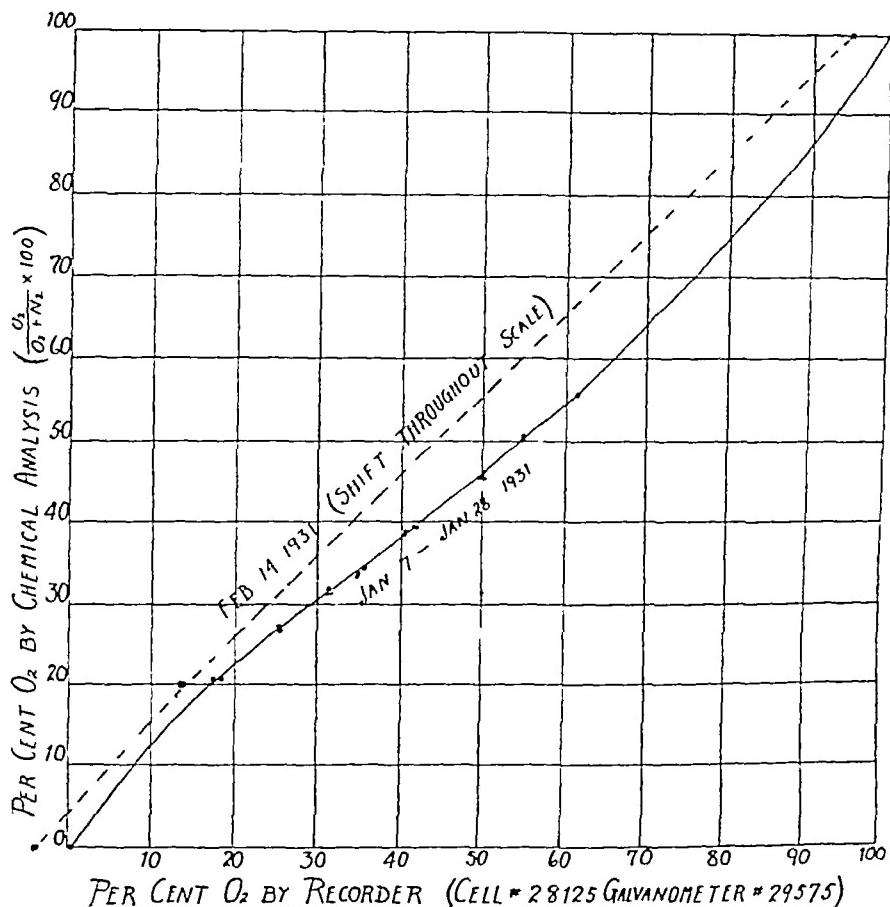


FIG 7 CALIBRATION CURVE FOR OXYGEN

Calibration curve for single by-pass cell no 28125, showing relation between the per cent scale deflection shown by the recorder and the volume per cent concentration of oxygen in the oxygen-nitrogen mixtures tested. The relation is not linear. The broken curve is drawn through three points obtained after a shift in the scale readings, which is discussed in the text.

compensating plugs of the two cells were adjusted so as to balance the two bridges (as indicated by zero galvanometer deflection) when both compartments of the double by pass cell contained carbon dioxide free and moisture free room air, and when the single by pass cell contained dry commercial nitrogen in its analysis compartment. The sensitivity of each cell was then damped by inserting series resistance in the two circuits connecting the cells to the galvanometer. The resistance was adjusted for the double by pass cell until the deflection was equivalent in tenths of a full scale to the per cent of carbon dioxide present.

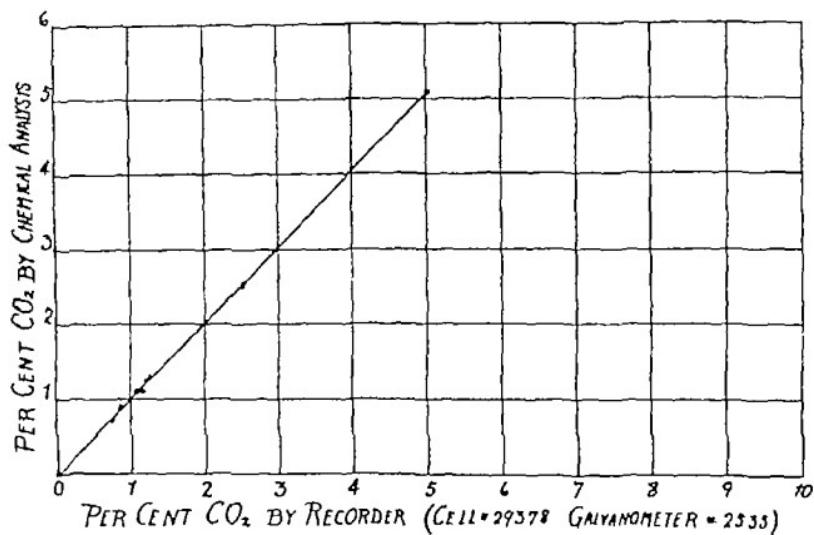


FIG. 8 CALIBRATION CURVE FOR CARBON DIOXIDE

Calibration curve for double by pass cell no. 29378 showing relation between per cent scale deflection and the volume per mille concentration of carbon dioxide in the gas mixtures tested. The relation is seen to be linear throughout the range tested.

in a given oxygen carbon dioxide mixture and for the single by pass cell, until oxygen from a cylinder gave full scale deflection. The deflections obtained from both cells with gas mixtures of lower carbon dioxide and oxygen content were then compared with the values shown by chemical analysis and the two cells were thus calibrated throughout the range for which they were to be used (3).

The calibrations were repeated several times after the instrument had been installed at the hospital, the curves obtained (Figs 7 and 8) being closely similar to those given by Hamilton (3).<sup>2</sup> Before each calibration test, the zero point of the galvanometer was routinely checked, and the zero point of the cells was tested with nitrogen. In making the latter adjustment we originally used the compensating plug of the cell, described on page 605, but later, at Dr Hamilton's suggestion, adjusted the balance of the bridge by a slight turn of the slide wire screw provided in the upper part of the cell to change the ratio of the resistance of two arms of the bridge.

Slight variations are to be expected in the calibration curves for the single by-pass cell, depending on the impurities in the compressed nitrogen used in adjusting the zero reading and in the compressed oxygen used in adjusting the galvanometer series resistance for the cell. Indeed, readings taken with different cylinders of oxygen may differ by 1 or 2 per cent. The presence of these impurities (especially hydrogen in the electrolytically prepared commercial oxygen used in these studies, and the rare gases of the atmosphere for the various air mixtures) largely accounts for the S shape of the oxygen calibration curve. A corrected galvanometer scale can be attached to the instrument to read directly in parts of oxygen per 100 parts of oxygen-nitrogen mixture.

*Changes in the thermal and electrical balance of the oxygen cell.* Occasional shifts have been observed in the readings given by the single by-pass oxygen cell, the cause for which has not been conclusively determined. A general shift of this sort is shown in Figure 7, in which three check tests taken February 14, 1931, fall to one side of the calibration curve determined from tests on the same cell taken January 7-28, 1931. It will be seen that the instrument gave negative readings for nitrogen (using the same cylinder as in the previous tests) and that the readings for room air and oxygen were similarly displaced downward. Shifts amounting to 2 or 3 and sometimes to 6 or more scale divisions occurred from time to time for the oxygen cell, always in a downward direction. They were usually observed within a month after calibration or adjustment, and, if uncorrected, became progres-

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<sup>2</sup>The analytical check tests were carried out by Henry Israel

sively greater In order to correct the cell readings the Wheatstone bridge was restored to a condition of balance with nitrogen in the analysis compartment by means of the compensating plug at the base of the cell, or preferably by means of the adjusting screw for the bridge resistance wires situated at the top of the cell

Since persistent and progressive shifts of this type were not observed for the carbon dioxide cell it seemed possible that the oxygen cell was being contaminated by glycerine spray carried over by the gas stream from the glycerine bubbler which followed the carbon dioxide cell but preceded the oxygen cell in the path of the gas stream To eliminate this possibility the bubbler was moved (February 16, 1931) to a position following both cells instead of in between the two (Fig 6) On February 20, the oxygen cell was adjusted by means of the bridge resistance screw so as to read 0 for nitrogen It then registered 100 for oxygen from a cylinder and between 16 0 and 17 5 for room air, in agreement with the calibration curve determined a month previously About one month later (March 16) the oxygen cell was reading +0 2 with nitrogen 99 7 to 101 with oxygen and 16 6 with room air On March 17 the reading for room air was 15 5 and on March 20 it was 15 8 while oxygen from a cylinder showed 99 0 No appreciable shift had occurred

Check tests carried out ten days later revealed a marked downward shift of 5 scale divisions, which we were tempted to attribute to the accidental overheating of the oxygen cell to 180° F due to failure of the thermostat control on March 25 The cell was readjusted April 1 when the shift was observed, and continued to record accurately for over three weeks Tests with room air and nitrogen on June 3 and 13, however, again showed a downward shift of 3 scale divisions The cause remains unknown There has been no interruption of the thermostat control Since a scale shift has not occurred for the carbon dioxide cell and since the manufacturers claim that this difficulty has not been elsewhere observed for their single by pass cells it may perhaps be attributed to some unknown defect of our particular cell

*Time lag* The time lag associated with the recorder as set up at Harlem Hospital is about 8 to 10 minutes This lag is partly due to the volume of gas in the absorption and connection tubes and in the bubblers, and partly to the time required for thermal equilibrium to be

reestablished in the cells after a change in the gas composition. This factor must always be taken into account in interpreting the record, as well as when the instrument index is used as a guide during the purging process or in detecting and compensating oxygen loss due to opening of doors.

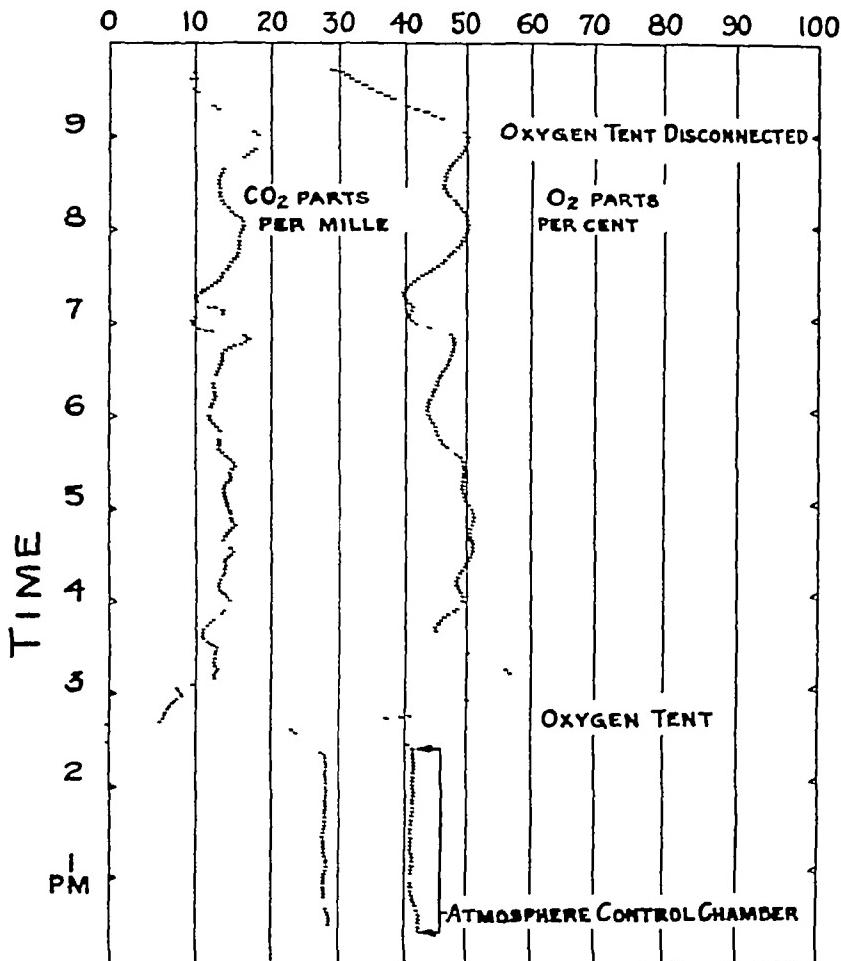


FIG 9 SAMPLE OF RECORD SHEET OF OXYGEN AND CARBON DIOXIDE CONCENTRATION IN OXYGEN CHAMBER AND IN OXYGEN TENT AT HARLEM HOSPITAL

Time in hours recorded vertically. Concentration of gas recorded horizontally in terms of volume parts per cent for oxygen uncorrected by calibration curve (recorded at right on chart), and volume parts per mille for carbon dioxide (recorded at left).

*Interpretation of chart* Figure 9 shows a typical sample of recorder chart, obtained when the instrument was connected to the oxygen chamber (12.20 to 2.20 P.M.) and to an oxygen tent (2.40 to 9 P.M.). The time is recorded vertically in hours, each hour being subdivided into 20-minute periods. The concentration of gas is recorded horizontally. The oxygen curve represents volume parts per cent of oxygen-nitrogen mixture (uncorrected for the scale error of the instrument). This curve is recorded on the original record in red. The carbon dioxide curve (recorded in black) represents volume parts per mille of total dry gas mixture. There is no scale correction for this curve.

#### SOURCES OF ERROR

Faulty readings may be caused by one or more of the following factors:

1 *Disturbance of the mechanical zero of the galvanometer* The galvanometer lever is adjusted by means of a screw on top of the recorder case, and should be set so as to read zero when no current is flowing through the galvanometer.

2 *Disturbance of the bridge current* The instrument is very sensitive to changes in bridge current differences of one or two milliamperes being registered as a change in the apparent gas concentration. With proper daily care of the batteries, however, and adjustment of the two bridge rheostats after the daily reversal of the battery switches, the current should not vary appreciably from the correct value of 240 milliamperes.

3 *Leaks in the pipe connections* Care must obviously be taken to avoid leakage of air into the cells. To this end the equipment should be assembled with as few tube connections as possible.

4 *Exhaustion of the absorbents*

5 *General shift throughout the cell readings* This error may be caused by a disturbance of the mechanical zero of the galvanometer, or by a disturbance of the thermal and electric balance of the cell.

6 *Inaccurate temperature control by the oxygen cell thermostat*

#### OPERATING REQUIREMENTS

The routine care of the batteries, suction pump, absorption tubes and recording mechanism can be assigned to an orderly but the instru-

ment should be operated under the supervision of a technician familiar with chemical and electrical equipment. It is essential to carry out check tests on the instrument at regular intervals.

#### *Routine care*

1 *Absorption tubes* (one-half hour to one hour per week) Under our working conditions it has been found necessary to change the absorbents at least twice a week and sometimes daily. The calcium chloride must be renewed when the reaction has extended through one-fourth the length of the tube. Since no visible change occurs in the soda lime after reaction, a small CO<sub>2</sub> indicating tube containing fresh sodium hydroxide flakes can be placed in the line that includes the soda lime, after the calcium chloride tube, or a few flakes may be placed at the outlet end of this calcium chloride tube. Penetration of moisture will be indicated by liquefaction and fusion of the flakes, and penetration of carbon dioxide by change of the pearly caustic to chalky carbonate.

2 *Current control* (five minutes daily) The batteries should be tested daily with a hydrometer, to insure a specific gravity of at least 1.215 for the battery connected to the recorder, and to prevent over-charging of the duplicate battery connected to the 110-volt circuit. We reverse the battery switches daily, and, since our rate of charging somewhat exceeds our rate of discharging, we occasionally give each battery a twelve-hour rest period. With these precautions, the bridge current remains very steady, and seldom requires adjustment. When alternating current is available a single battery with a trickle charger can be used.

3 *Recorder* The clockwork must be wound once or twice a week, the inked ribbon reversed once every few weeks, and the chart replaced once a month.

4 *Suction pump* (two hours quarterly) If the recorder is to be used with high concentrations of oxygen, the rotary pump should not be lubricated with oil because of the resulting fire hazard. Glycerine is an unsatisfactory lubricant, tending to char, and clog the bearings. Aquadag, an aqueous suspension of graphite, can be used, but similarly causes the bearings to become clogged from time to time. We have found undiluted graphite to be the most dependable lubricant. The pump must be cleaned out once every few months.

*Periodic tests*

It is recommended that the supervising technician carry out the following tests. All tests should be preceded by routine examination of the mechanical zero of the galvanometer of the bridge current and of the temperature of the oxygen cell thermostat.

1 Once a week the recorder should be allowed to analyze a sample of ordinary room air in which case the carbon dioxide should read 0 to 0.1 per cent and the oxygen should read 20 to 21 per cent on the corrected scale (or 16 to 18 per cent on the uncorrected scale). This test is useful as an indication of a general displacement or shift in the recorder readings for one or both cells (see paragraphs 1 and 5 of the section headed *Sources of Error*). It gives little or no information regarding leakage since the air in the cells is closely similar to that surrounding the pipes and connections.

2 If one of the cells gives a correct reading but the reading for the other cell is appreciably displaced from the correct value, this cell should be tested for a general shift (see pages 620 and 621 and paragraph 5 of the section headed '*Sources of Error*'). The oxygen cell which was the cell for which we observed an occasional unexplained shift can be tested most readily by stopping the suction pump and forcing dried nitrogen or dried oxygen through the cells at the usual rate (80 to 120 bubbles per minute through each bubbler, or less than one liter per minute for the combined streams) from a cylinder provided with a hospital flow regulator. If no shift has taken place, and the error was due to leakage into the oxygen cell under suction the oxygen cell of the recorder will then register 0 with nitrogen and 100 with oxygen. If a shift has taken place, the readings with room air, with oxygen and with nitrogen will show parallel discrepancies from the calibration curve, as are shown in Figure 7 by the oxygen readings for February 14. It is advisable to notify the manufacturers if the cell readings show a tendency to shift, rather than to correct the shift whenever discovered by adjustment of the compensating plug or the bridge resistance of the single by pass cell.

3 At least once a month the recorder should be checked by chemical analysis of the air sample. The Haldane absorption burette is accurate only up to 40 per cent of oxygen. We have obtained satis-

factory results with the absorption apparatus shown in Figure 10, which is adapted to oxygen and carbon dioxide analysis to within  $\pm 0.1$  per cent, the oxygen range extending from 0 to 65 parts per 100 parts of oxygen-nitrogen mixture. This apparatus is portable and

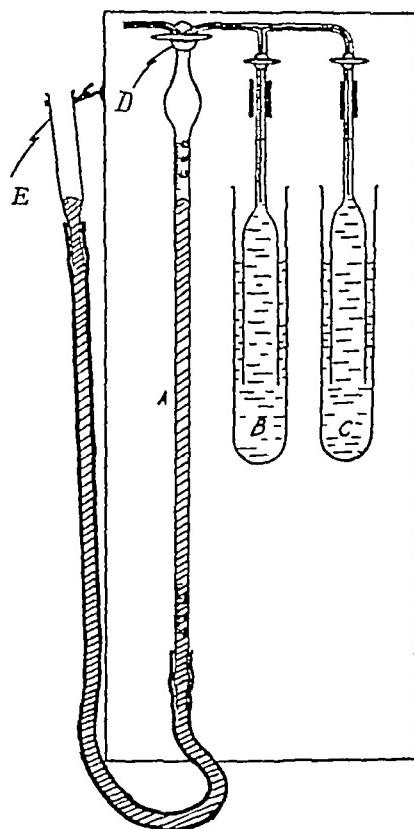


FIG 10 GAS ANALYSIS APPARATUS FOR CARBON DIOXIDE AND OXYGEN, FOR CONCENTRATIONS FROM 0 to 65 VOLUMES PER CENT

A, burette holding 12 cc of gas, graduated 0-65 per cent, in 0.2 per cent divisions B, absorption pipette for oxygen C, absorption pipette for carbon dioxide D, three-way stopcock E, levelling bulb with mercury

inexpensive. We use a 20 per cent solution of potassium hydroxide for the carbon dioxide, and for oxygen absorption sodium hydrosulphite, the reaction being accelerated by sodium anthrahydroquinone  $\beta$ -sulphonate, as recommended by Van Slyke (10).

The readings on the corrected scale should agree with the chemical

analysis to within  $\pm 0.15$  per cent for carbon dioxide and  $\pm 1.5$  per cent for oxygen.

If the instrument is inaccurate by 0.2 per cent or more for the carbon dioxide readings or by 2.0 per cent or more for the oxygen readings, the cells should be tested for a shift throughout the scale, using room air and tank oxygen or nitrogen, as discussed above in paragraphs 1 and 2. If parallel discrepancies do not appear throughout the scale, the error is probably due to exhaustion of the absorbents or to leakage. The chemical check tests should then be repeated after renewing the absorbents, resealing all permanent connections with paint, and renewing all rubber connections. It should be stressed that leakage of outside air into the cells will not result in a positive error for the oxygen readings unless the air sample under test contains less oxygen than ordinary room air. The presence of water vapor in the oxygen cell (exhaustion of the drying agent) may, however, slightly raise the apparent oxygen reading (2). The presence of carbon dioxide in the oxygen cell (exhaustion of the decarbonating agent when the gas sample is rich in carbon dioxide) will lower the apparent oxygen reading. Leaks into the double by-pass carbon dioxide cell may cause either a positive or a negative error.

#### USE OF KATHAROMETER FOR INDICATION OF OXYGEN AND CARBON DIOXIDE IN AIR

A Katharometer capable of indicating from 0 to 10 parts of carbon dioxide in air was put at our disposal for preliminary study through the courtesy of the Cambridge Instrument Co. Our tests showed that this single by-pass thermal conductivity instrument can also be adapted as an oxygen in air indicator.

Since the Katharometer is not equipped as is the Engelhard instrument with a mechanical means for balancing the two compartments of the thermal cell nor with a bridge slide-wire adjustment, the galvanometer will show a zero deflection only when analysis and reference compartments contain thermally identical gas mixtures. With dry room air in the reference compartment, and the galvanometer leads reversed from the usual position for carbon dioxide indication, the instrument indicates an excess in oxygen concentration over the normal 21 per cent. The sensitivity in terms of scale deflection per volume

per cent change in concentration is for oxygen about 1/10 of that for carbon dioxide. Thus, whereas full scale deflection in one direction is registered for a mixture containing the normal oxygen-nitrogen balance plus 10 per cent carbon dioxide, tank oxygen, containing 79 per cent more oxygen than ordinary air, gives between 70 and 80 per cent of a full scale deflection in the opposite direction. Oxygen deficiencies (concentrations of less than 21 per cent) will show a positive deflection when the galvanometer is connected as for carbon dioxide indication.

It will be seen that the instrument can be made to function as both oxygen and carbon dioxide indicator for mixtures in which both gases vary independently. To this end the reference compartment is filled with dry room air, the galvanometer leads are connected to a reversing switch, and consecutive tests are carried out with a gas sample that is both dried and decarbonated and with another sample of the same gas that is dried but not decarbonated. The concentration of oxygen, in volume parts per cent of the oxygen-nitrogen mixture, will be given by adding 21 to the number of per cent scale divisions deflected in the first galvanometer reading, counting the galvanometer reading as positive when the leads are connected as to indicate pure oxygen, and negative when the leads are reversed as to indicate pure nitrogen. The carbon dioxide concentration of the mixture, in parts per mille, will be given by subtracting the second reading from the first reading, using the same convention as to the sign of the galvanometer reading. For example, if the first reading should be -5, and the second reading -45 per cent of a full scale deflection, the oxygen concentration would be 21 - 5, or 16 per cent, and the carbon dioxide concentration would be -5 - (-45), or 40 parts per mille.

We did not attempt to calibrate the katharometer for oxygen determinations nor to check closely the constancy and reproducibility of its readings.

Our tests with the katharometer led us to question the present method of attempting to balance the water vapor in the two compartments of the thermal cell by saturating the air samples in both compartments, instead of by eliminating the water completely. The instrument is used largely for carbon dioxide determinations of alveolar air, which is presumably saturated with water vapor at about 35° C. The

closed reference compartment, on the other hand, is provided with a moist wick which maintains a saturated atmosphere at an unknown temperature, probably very close to room temperature. The temperature effect on the saturation concentration of the two air samples appears to have been overlooked.

In determining the zero reading of the cell the analysis compartment is fitted with ordinary room air, moistened by bubbling through water. The resulting partial pressure of water vapor will depend on the temperature and on the degree of saturation obtained by the bubbling method, and it may readily vary from 15 to 24 mm. of mercury, corresponding to from 2 to 3 per cent by volume. The water vapor in the sealed compartment with the moist wick will correspond to that in the analysis compartment only if the temperature and the degree of saturation coincide. Certainly the partial pressure of water vapor in a sample of alveolar air differs from that of room air. Assuming the sample to be saturated with moisture at 35° C., the water vapor would exert a pressure of 41 mm. of mercury, and its concentration by volume be about 5.5 per cent. If variations of about 1 or 2 per cent in the volume concentration of water vapor affect appreciably the thermal conductivity of air, the katharometer readings would be in error unless water were completely removed from both compartments. At the same time, the thermal effect of water vapor, as estimated by two independent methods appears to be definitely appreciable.

From the data of Gruss and Schmuck (2) a 7 per cent water air mixture has at 80° C. a thermal conductivity equal to that of dry air multiplied by the factor 1.02. Eucken's value for the thermal conductivity of dry air at 100° C. is  $71.9 \times 10^{-4}$  (Table 1). On the assumption that the same ratio holds at this temperature, a 7 per cent water air mixture at 100° C. would have a thermal conductivity of  $73.3 \times 10^{-4}$ . If we make the further assumption of a linear relation between thermal conductivity and water vapor concentration within the range 0 to 7 per cent, the thermal conductivity of air will be increased by  $1.4 \times 10^{-4}/7$  or by  $0.2 \times 10^{-4}$  per per cent increase in water vapor for this range. The difference between the thermal conductivity of dry air and of carbon dioxide is  $22.3 \times 10^{-4}$  at 100° C. (Table 1). If we assume a linear relation between thermal conductivity and concentration for carbon dioxide in air mixtures, we find that the thermal conductivity of air is decreased by  $22.3 \times 10^{-4}/100$  or by  $0.2 \times 10^{-4}$  per per cent increase in carbon dioxide. Therefore the effect of differences in water vapor concentration in terms of volume per cent

should for this region be of the same order of magnitude as that of differences in carbon dioxide concentration.

This estimate is in agreement with the results of a test in our laboratory on the effect of water vapor in low concentrations, using the Engelhard double by-pass (carbon dioxide) cell. Since water vapor, in concentrations up to about 20 per cent, increases the thermal conductivity of air while carbon dioxide decreases it, the galvanometer leads to the Wheatstone bridge were reversed from their normal position for carbon dioxide. Dried room air was admitted to both compartments of the cell until a constant galvanometer reading had been obtained. The calcium chloride tube was then disconnected from the air stream leading to the analysis compartment and when conditions had again become steady a second reading was taken. This reading was exactly 10 scale divisions higher than the first, a displacement equal in magnitude (though opposite in sign) to that caused by an increase in carbon dioxide from 0 to 1 per cent. Wet and dry bulb thermometer readings were 57.4 and 69.2 respectively, indicating a partial pressure of 8.6 mm of mercury due to water vapor, or a concentration of about 11 per cent water vapor.

Since the effect of water vapor in low concentrations thus appears to be of about the same order of magnitude as that of carbon dioxide, it is not clear how the neglect of moisture differences in the use of the katharometer for alveolar air studies can be compatible with accurate results.

#### SUMMARY

- 1 Continuous gas analysis is recommended as an aid in clinical atmosphere control.
- 2 A review is presented of the theory of quantitative gas determinations by the thermal conductivity method.
- 3 The difficulties attending the application of the thermal conductivity method of analysis to oxygen and carbon dioxide in air mixtures are discussed.
- 4 Tests are presented leading to the adoption of a double by-pass thermal cell for carbon dioxide in air measurements, and of a single by-pass cell for oxygen in air measurements.
- 5 The Engelhard carbon dioxide and oxygen recorder is described in detail.
- 6 Sources of error are enumerated.
- 7 Operating requirements and control tests are listed and described.

8 The adaptability of the "katharometer" for oxygen analysis is discussed

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## AN ELECTROCARDIOGRAPHIC STUDY OF THE HEART IN LOBAR PNEUMONIA<sup>1</sup>

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### INTRODUCTION

Numerous electrocardiographic studies have been made in rheumatic fever diphtheria and other acute infectious diseases (1, 2, 3 4, 5), but a search of the literature yields relatively little information on the changes in the heart depicted by the electrocardiogram in lobar pneumonia. Cohn and Jamieson (6) in a report on the action of digitalis in this disease discussed the tracings obtained from fifty patients not treated with digitalis. Although certain facts can be ascertained from a study of such a group it is nevertheless true that a much larger series gives a more comprehensive picture. Furthermore, Cohn and Jamieson selected largely the mild cases to serve as controls, while those who were severely ill received digitalis. Therefore, it seemed advisable to study a large unselected group of patients to whom no digitalis had been given.

The data forming the basis of this report were obtained during the course of an investigation on the action of digitalis in lobar pneumonia. The method of selection of cases, the routine established, and the precautions observed are given in detail by Wyckoff, DuBois and Woodruff (7). Suffice it to say, that an absolutely unprejudiced random sample was obtained for both digitalis treated and untreated groups. Electrocardiograms were taken daily during the acute or febrile period of the disease and every other day during convalescence.

<sup>1</sup> Read before the New York University Medical Society October 4 1930 and the Section of Medicine New York Academy of Medicine December 16 1930

A control tracing was taken on all treated cases before any digitalis was administered. During the three years of the study 7,480 electrocardiograms were obtained on 975 patients, of whom 562 received no digitalis. On 101 patients (10.4 per cent) only one tracing, and on 89 (9.1 per cent) two tracings were taken. For the remaining 785 (80.5 per cent), the average number of electrocardiograms per patient was nine. During the first year a Cambridge portable machine was used, and in the second and third years, a Hindle Mobile No. 3.

Since this study deals with the electrocardiographic findings, uninfluenced by treatment (notably digitalis), occurring in lobar pneumonia, the analyses which follow are based on the tracings obtained from 562 patients who received no digitalis and on the control tracings from 413 digitalis treated cases, taken before any digitalis had been administered. The study of the abnormal basic rhythms however, is based, as will be explained later, on all electrocardiograms taken on the 975 digitalis treated and untreated cases.

The material to be presented lends itself to division into three main groups. They are (1) heart rate, (2) abnormalities of rhythm and (3) abnormalities of conduction. The abnormalities of rhythm will be further subdivided into (a) the abnormal basic rhythms and (b) interruptions of the normal basic rhythm.

#### *Heart rate*

Clinicians have for years attached great importance to the heart rate in lobar pneumonia. A very rapid rate is usually associated with a severe infection and is generally regarded as indicative of a poor prognosis. Bullowa (8) has used this experience in establishing a severity rating in lobar pneumonia. He attached relative numerical values to degrees of cyanosis, respiratory rate, bacteremia, etc., as well as to the heart rate, in an attempt to forecast the prognosis. The increased severity of the disease with the higher heart rates was acknowledged by a deduction of five points from the patient's rating for every increase of ten heart beats above 110 per minute.

No one would question that the electrocardiogram is the most reliable source for the determination of the ventricular rate. Our data therefore, afford an excellent opportunity for the study of the heart rate in lobar pneumonia. Occasionally, the first tracing shows

a slightly higher rate than subsequent ones, probably due to the apprehension of the patient. Later, the patient is not alarmed and it is possible to record a somewhat lower heart rate which varies between rather narrow extremes.

It can be seen from the curves of individual patients in Figures 1,

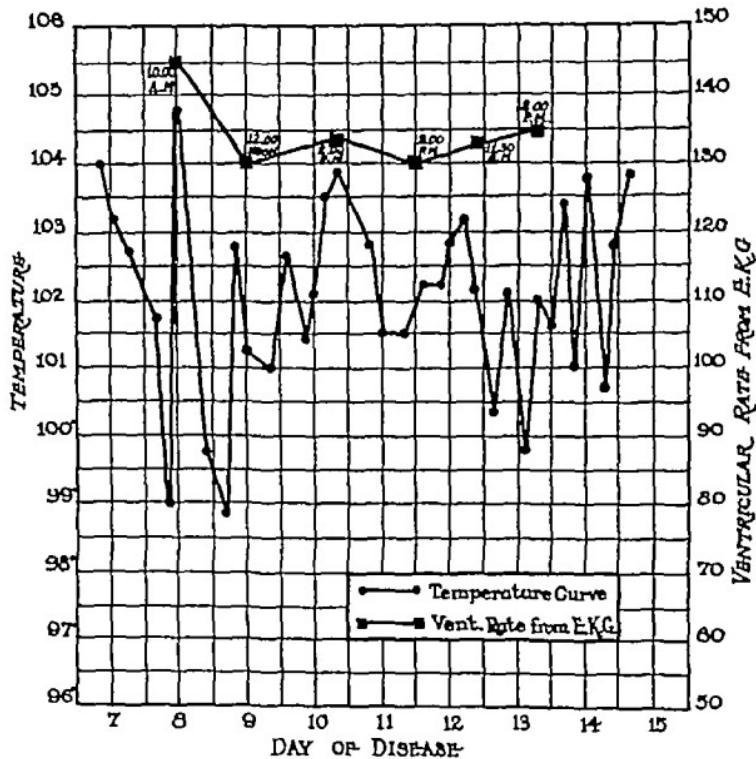


FIG 1 MALE AGE 36

Illustrates (1) a rapid heart rate during fever (2) after the first tracing the remarkable constancy of the heart rate from day to day in spite of fluctuations of temperature.

*Note*—In all figures the day of disease, as represented on the abscissae is calculated from the history of the onset.

2 and 3 that the heart rate varies within very narrow limits and that it remains within these narrow limits from day to day during the febrile period even in the presence of fluctuations of temperature

That such variations of heart rate are slight in a large proportion of cases is shown in Figure 4, in which the maximum range of heart rate during the febrile period for cases with sinus rhythm is plotted against the respective incidence of each. It is striking that 27 per cent showed a variation in rate of less than ten beats per minute, and that 66 per cent had a maximum range of less than twenty-one per minute.

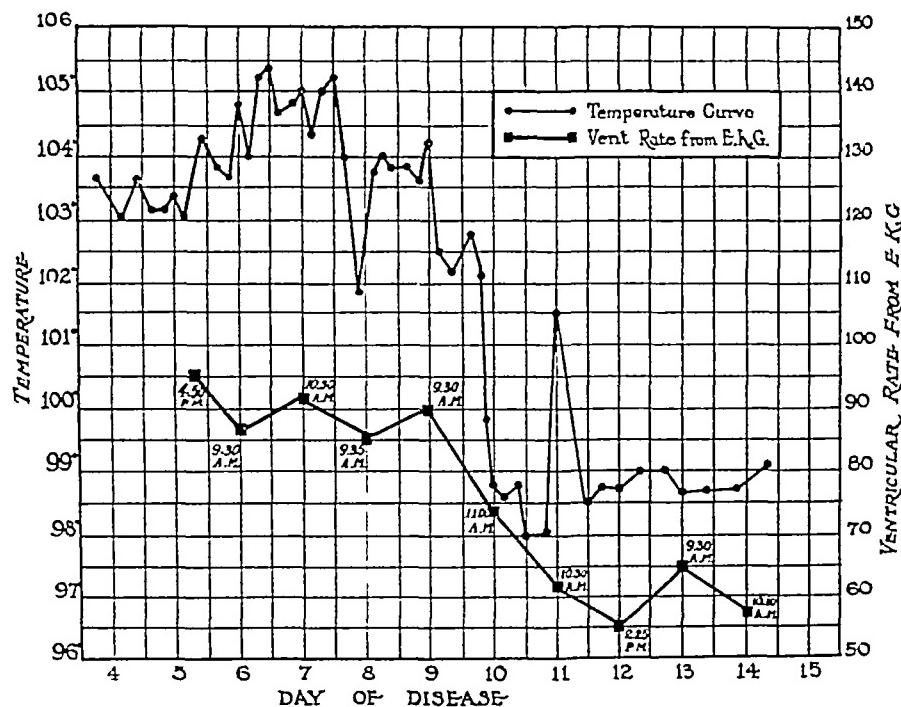


FIG. 2 MALE, AGE 40

Illustrates (1) a relatively slow heart rate during fever, (2) the constancy of the heart rate during fever as calculated from the electrocardiogram, (3) sinus bradycardia after the crisis.

Where a large variation was present, it was found to be associated with one of the following conditions (1) a premortal rise in heart rate (2) a fall in temperature by lysis (since the range in heart rate includes the entire febrile period), and (3) a fast heart rate in the first tracing, as noted above.

It may seem possible that the truth is not reached by observations made only once in a twenty-four hour period, that variations in heart

rate during the period between electrocardiographic observations may be missed and that the constancy in heart rate results only from the conspiracy of chance observations. Since the great majority of cases showed a relative constancy of the heart rate, this supposition is not justified. Furthermore, in six cases, whose average heart rates varied from 84 to 144 beats per minute, electrocardiograms were taken every hour over a period of eight hours. During this period the heart rates

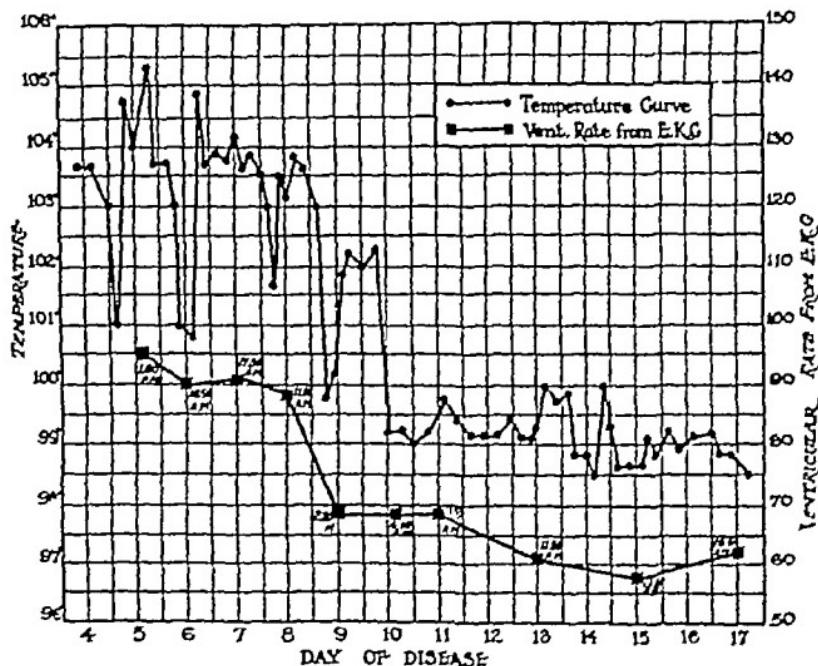


FIG. 3. FEMALE 16P 56.

Illustrates (1) a relatively slow heart rate during fever (2) the constancy of the heart rate during fever as calculated from the electrocardiogram.

varied from 6 to 10 beats per minute. In view of these facts, we feel satisfied that single daily observations of the heart rate (even in the presence of fluctuations of temperature) reveal the true variations. Our data furnish evidence, then, that in lobar pneumonia the variation in rate from day to day is slight.

In view of this fact the value of heart rate as one of the important

factors in the determination of prognosis becomes greatly increased especially early in the disease when other clinical signs may not be clearly defined. We have, therefore, studied the ventricular rate in

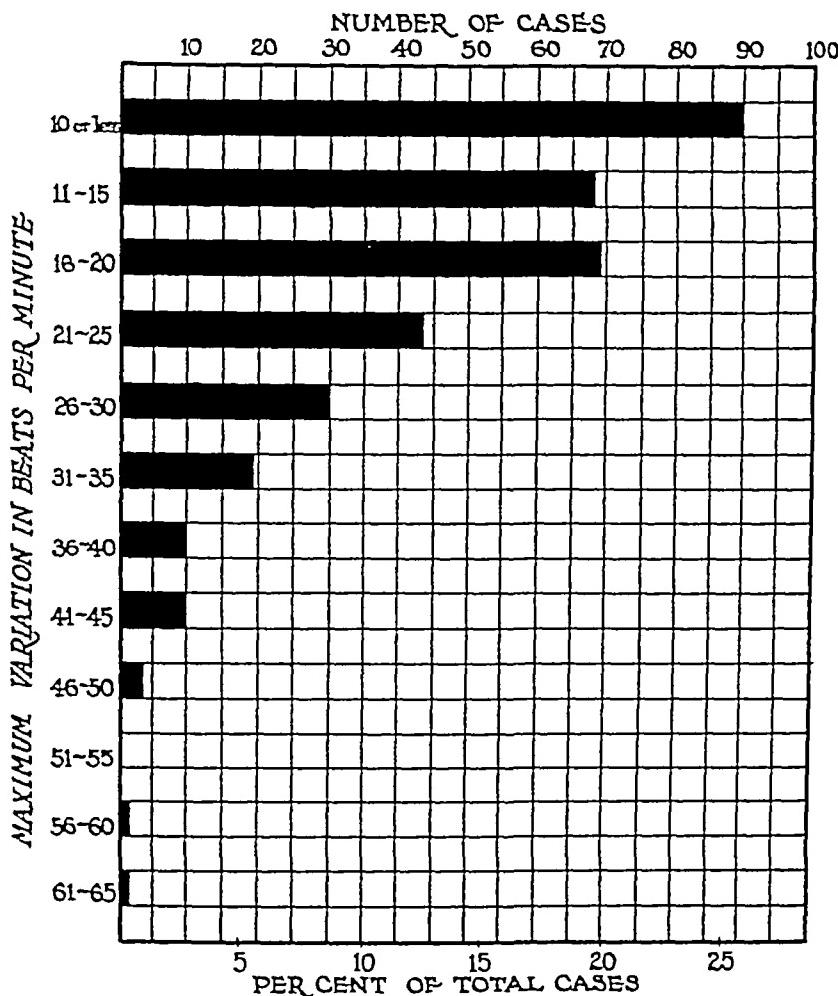


FIG 4

MAXIMUM RANGE IN HEART RATE DURING FEVER FOR 349 CASES WITH SINUS RHYTHM

all tracings taken before crisis, lysis, or death including the control electrocardiograms of patients treated with digitalis. A tabulation of these tracings was made according to the day of the disease on

TABLE I  
The incidence of a given heart rate according to day after onset of pneumonia

	Day of disease *						All			
	1, 2 and 3		4 and 5		6 and 7		8 and 9		Recovered	Died
	Recovered	Died	Recovered	Died	Recovered	Died	Recovered	Died		
Total number of electrocardiograms	154	61	295	146	296	148	179	124	924	479
Heart rates										
Under 110, per cent	57.7	39.3	65.8	30.8	64.5	27.9	65.1	21.7	64.0	28.6
Over 110, per cent	42.3	60.7	34.2	69.2	35.5	72.1	34.9	78.3	36.0	71.4

\* In all tables the day of the disease is estimated from the history of the onset.

which they were taken. This was done separately for the recovered and fatal cases, and the results are shown in Table I. It may be stated generally that nearly two-thirds of the electrocardiograms taken on patients who subsequently recovered showed ventricular rates under 110 per minute regardless of the day of the disease on which the rate was recorded, and conversely, that more than two-thirds of the tracings obtained from patients who later died showed ventricular rates over 110 per minute. It may also be seen that the poor prognosis of a high ventricular rate becomes worse as it occurs later in the disease.

This conclusion suggested to us that we might arrange the data in the form of a life expectancy table (Table II). Although this is based on the number of electrocardiograms rather than on the number of patients, it clearly indicates the general trend of mortality for the different rate groups throughout the febrile period.

It is noteworthy that a fair number of tracings (11.3 per cent) show heart rates of 70 to 89 per minute during the period of fever. It might be assumed that in these cases the temperature was proportionately low, but as a matter of fact, in many the temperature was very high. We have selected the charts of two patients showing this condition and have reproduced them in Figures 2 and 3.

Exceedingly slow heart rates due to sinus bradycardia were not uncommon after the temperature had become normal. At some time during convalescence, heart rates of less than sixty per minute were noted in 105 patients (32 per cent) not treated with digitalis who recovered. Nineteen patients (4.8 per cent) had rates between 40 and 49, and one showed a sinus bradycardia of 38 per minute in one tracing.

From our data we have thus far demonstrated that the heart rate is remarkably constant from day to day during the febrile period. We have also shown that with higher heart rates the expectancy of life diminishes, this relationship being even more pronounced in the later days of the disease. We have noted the occurrence of heart rates slow in proportion to fever, and of sinus bradycardia during convalescence.

TABLE II  
*Expectancy of life for rate groups by day of disease*

Heart rates	Day of disease						All
	1, 2 and 3	4 and 5	6 and 7	8 and 9	Recovered	Number of tracings	
Number of tracings	Recovered per cent	Number of tracings Recovered per cent	Number of tracings Recovered per cent	Number of tracings Recovered per cent	Recovered per cent	Number of tracings	Recovered per cent
70-89	16	61	59	94.9	41	82.9	177
90-109	97	78.3	77.0	78.1	102	80.5	551
110-129	83	67.5	149	57.9	154	55.2	78.1
130-149	15	46.7	44	27.3	51	39.2	49.5
150 plus	4	50.0	8	25.0	7	0.0	56.6
						6	31.6
						0.0	25
							16.0

*Abnormalities of rhythm*

*a Abnormal basic rhythms* Auricular fibrillation and flutter in lobar pneumonia have received considerable attention, particularly from the standpoint of prognosis and treatment. In our series of 975 cases (digitalis treated and untreated) auricular fibrillation and flutter occurred in 35 instances (3.6 per cent). In addition to auricular fibrillation and flutter, we have included paroxysmal tachycardia and wandering pacemaker as disturbances of the basic rhythm of the heart. These irregularities were recorded by the electrocardiograph in 51 cases. With more frequent tracings a higher incidence would probably have been found as all of these arrhythmias were paroxysmal in nature with the exception of ten cases of persistent auricular fibrillation.

Because the number of cases in each category is so small and because the incidence is found to be approximately the same for digitalis treated and untreated groups (Wyckoff, DuBois and Woodruff (7)), in dealing with these abnormal basic rhythms we have included also the electrocardiograms of patients who received digitalis.

In the first analysis of abnormal basic rhythms (Table III), there

TABLE III  
*Incidence of abnormal basic rhythms in 975 cases \* and associated mortality*

Type of arrhythmia	Number of cases	Incidence per cent	Number died	Mortality per cent
Auricular fibrillation	27	2.8	17	63.0
Auricular flutter	10 †	1.0	7	70.0
Paroxysmal tachycardia	6	0.6	4	66.7
Wandering pacemaker	8	0.8	4	50.0
Total—all types	51	5.2	32	62.8

The mortality of the entire series excluding the patients with abnormal basic rhythms (924 cases) was 34.3 per cent.

\* In this table both the digitalis treated and untreated cases are included.

† Two of the cases of auricular flutter also showed fibrillation and have been included in both groups.

are two outstanding facts to be noted, namely, their surprisingly low incidence and their consistently high mortality. The mortality for the various types of arrhythmias ranges from 15 per cent to 35 per cent higher than the gross mortality for all cases with sinus rhythm, and

the average mortality for all the arrhythmias is 28.5 per cent higher. Since in the general series of pneumonia patients the older age groups show a much higher mortality than do the younger, a difference especially striking above and below forty, the very high figure for the arrhythmias might be thought to be due to the fact that a large proportion of them occurred in the older age groups. It is indeed true that the incidence of arrhythmias (Table IV) in patients over forty is

TABLE II

*The incidence and mortality of cases with abnormal basic rhythm compared with sinus rhythm for the age groups above and below forty years \**

	Abnormal basic rhythms 51 cases		Sinus rhythm 924 cases	
	Under 40	Over 40	Under 40	Over 40
Number of cases	9	42	446	478
Incidence per cent	17.6	82.4	48.3	51.7
Mortality per cent	55.6	64.3	22.2	45.7

\* In this table both the digitalis treated and untreated cases are included.

nearly five times that in patients below that age, while the incidence of sinus rhythm is practically the same in both age groups. However, in cases with sinus rhythm the mortality rate over forty years of age is twice that under forty, whereas the occurrence of abnormal basic rhythms raises the mortality of both age groups and makes them more nearly equal. This comparison indicates a marked increase in mortality when disturbances of basic rhythm occur regardless of the age of the patient. It was thought possible that arrhythmias developed chiefly in the more severe types of pneumonic infections. Therefore, a comparative study of the incidence of the pneumococcal types was made and the distribution found to be essentially the same for these arrhythmias as for sinus rhythm. One must therefore conclude that the development of an abnormal basic rhythm in the course of pneumonia seriously affects the prognosis by its own influence.

b *Interruptions of the basic rhythm.* Premature contractions are to be considered as interruptions of the basic rhythm of the heart. It is obvious that for their analysis electrocardiograms taken on digitalis

treated cases must be excluded. The different types of premature contractions have been studied separately and their incidence and mortality are shown in Table V. The occurrence of ventricular

TABLE V  
*Incidence and mortality of cases with premature contractions*

Type of premature contraction	Number of cases	Incidence per cent	Number died	Mortality per cent
Ventricular only	32	5.7	10	31.3
Auricular or nodal alone, or in combination with ventricular	33	5.9	14	42.4
Total—all types	65	11.6	24	36.9

premature contractions alone does not seem to influence the prognosis, for the mortality figure (31.3 per cent) of cases showing this type of premature contraction is almost identical with that of the entire series (31.2 per cent). When the auricular or nodal premature contractions occur alone, or in combination with ventricular premature contractions, the mortality rate is somewhat higher. An explanation for this is suggested by the data in Table VI, in which the association

TABLE VI  
*Association of abnormal basic rhythms with premature contractions*

Type of premature contraction	Number of cases	Those associated with abnormal basic rhythms	
		Number	Per cent
Ventricular only	32	2	6.2
Auricular or nodal alone, or in combination with ventricular	33	6	18.2

of the various types of premature contractions with disturbances of the basic rhythm is tabulated. The incidence of ventricular premature contractions in association with arrhythmias is low as compared with that of auricular and nodal premature contractions. This fact might account for the slightly higher mortality in the latter group. It may be of interest to note that of the 33 cases showing auricular or nodal premature contractions, 24 occurred alone and 9 in combination with the ventricular type.

It is evident that when the basic rhythm of the heart is altered the prognosis is not nearly so good as when a sinus rhythm is continuously present. However, the occurrence of ventricular premature contractions alone should not be regarded as a poor prognostic sign.

#### *Abnormalities of conduction*

The occurrence of auriculoventricular block in patients receiving no digitalis is not rare. Using the criteria of Lewis and Gilder (9), that the upper limit of the normal P-R interval is 20 second, in our series of 562 cases we noted prolongation of auriculo-ventricular conduction time in 51 (9.2 per cent), dropped beats in three (0.5 per cent), and complete dissociation in none. Cohn and Jamieson (6) did not mention the occurrence of heart block in fifty digitalis control cases, but they were primarily interested in the maximum change in the P-R interval. They found no case in which the P-R interval increased more than 0.4 seconds during fever. In two cases they found a decrease exceeding that figure. In order to make our figures comparable to a certain extent with those of Cohn and Jamieson, we have determined the maximum range in the P-R interval for our cases of prolonged conduction time (Table VII). Over 85 per cent showed a change of 0.4 second or more. In Table VIII the minimum

TABLE VII  
*Maximum range of P-R intervals in 51 cases of prolonged conduction time*

	Maximum range in seconds								Unknown *	Total
	.03	.04	.05	.06	.07	.08	.09			
Number of cases	5	17	7	11	4	5	1	1	51	
Incidence, per cent	9.8	33.3	13.7	21.6	7.8	9.8	2.0	2.0	100.0	

\* One tracing only.

TABLE VIII  
*Minimum P-R intervals in 51 cases of prolonged conduction time*

	Length of minimum P-R interval in seconds									Total
	.14	.15	.16	.17	.18	.19	.20	.21	.22	
Number of cases	1	0	14	7	15	3	9	0	2*	51

\* One of these cases had one tracing only.

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P-R intervals are given, and it may be seen that all but two cases showed at some time what may be considered a normal P-R interval. From Tables IX and X it is shown that in 50 per cent of the cases

TABLE IX  
*Relation of increased I-I conduction time to fever in 562 cases*

	I febrile period	Afebrile period	Total
Number of cases	11	40	51
Percentage of cases	21.6	78.4	100
Number died	3	0	3
Number later showing normal P-R interval	5	20	25
Percentage restored to normal P-R interval	45.5	50.0	49.0

TABLE X  
*Incidence of increased I-V conduction time according to days after crisis*

Days after crisis	Prolonged P-R interval		Restored to normal P-R interval	
	Number of cases	Incidence in 40 cases per cent	Number of cases	Percentage per cent
1-2, 3	22	55.0	11	50.0
4-5, 6	7	17.5	1	14.3
7-8, 9	6	15.0	5	83.3
10-11, 12	1	2.5	1	100.0
13-14	4	10.0	2	50.0
All days	40	100.0	20	50.0

Note. There were no cases in which the P-R interval first became prolonged later than the 14th day after the crisis.

with increased A-V conduction time, the P-R interval was restored to normal at some time during our observation. This seems to indicate that the changes in conduction are definitely related to the disease.

In certain other diseases, namely, rheumatic fever and diphtheria, electrocardiographic changes found in the afebrile period are admitted as evidence that the myocardium has in some way been involved. Cohn and Jamieson (6) excluded from the analysis of their pneumonia series all cases in which the increase in conduction time occurred

during the postcritical period, whereas we have included them Cohn and Swift (1) in a report on the electrocardiographic evidence of myocardial involvement in rheumatic fever consider a prolongation of the P-R interval during the afebrile period, as well as during the febrile period, as evidence of myocardial involvement. Heart block in diphtheria occurs not only in the acute stages but later in the disease for instance Parkinson (10) reports a case of heart block occurring on the twenty third day. It therefore seems reasonable to consider as significant, similar changes occurring in lobar pneumonia. In Table IX we have compared the incidence of prolonged conduction time before and after defervescence. Although the major part (78 per cent) first shows a prolongation of the P-R interval after the temperature has reached a normal level, still in an appreciable number (22 per cent) block first appears during fever. In the latter group of eleven, nine cases had a maximum conduction time of 22 second and two, of 24 second.

In the postfebrile period auriculoventricular block occurs first very soon after the temperature has become normal. In Table X we have correlated the first appearance of increased conduction time in the convalescent period with the number of days elapsed after the crisis or, if defervescence was by lysis, with the number of days after the temperature became normal. Over half of the patients developed an increased P-R interval during the first three days of convalescence and in approximately one half of these cases the conduction times returned to normal while the patients were still under our observation.

The factor of heart rate must be considered in relation to prolonged auriculoventricular conduction time. Lewis and Cotton (11) have shown that after exercise, the increase in heart rate is accompanied by a reduction in the P-R interval. Cohn and Jamieson (6) noted that at the height of the fever in those cases of lobar pneumonia showing a rapid heart rate, there was a reduction in the auriculoventricular conduction time. We have correlated heart rate with increased conduction time, and in 34.7 per cent of our cases showing this change increasing P-R interval occurred simultaneously with slowing of the heart rate. However, in 52.1 per cent no such correlation could be found and in 13.2 per cent the P-R interval actually increased as the ventricular rate increased.

## 615 ELECTROCARDIOGRAPHIC STUDY OF HEART IN LOBAR PNEUMONIA

Various grades of intraventricular block, and in a few cases complete bundle branch block were found in both digitalis treated and untreated groups. In the first two years of this study measurements were not made of the duration of the QRS group and only frank cases of complete bundle branch block were noted. There were two such cases, one in each year. Both had the same degree of bundle branch block throughout and both died. It is possible that the block existed before the pneumonia developed and that the occurrence here is merely a coincidence. In the third year, accurate measurements of the width of the QRS group were made on every tracing. A total of nineteen cases (9.3 per cent) were found with QRS width exceeding 10 second during some part of the disease. The mortality in this group was only 26.8 per cent, which compares favorably with the gross mortality for the third year of 28.2 per cent. Eleven of these cases had a prolonged QRS group on the first tracing and continued to show intraventricular block on each subsequent tracing. It is likely that the intraventricular block antedated the pneumonia. Incidentally, only three of the eleven patients died, a surprisingly low mortality, considering the significance that is ordinarily attached to a wide QRS group. The other eight patients developed intraventricular block during the course of the disease. Two of these patients died. It is noteworthy that two patients did not develop an intraventricular block until after the temperature had become normal, indicating again that active changes in the heart do not terminate with the crisis.

### *Changes in the S-T segment and T wave*

An individual case in which there was marked elevation of the R-T segment with plateau-like T wave has already been reported by Shearer (12). A few cases showing similar changes of much less marked degree were also noted.

A change in direction of the T wave from an upright to an inverted position in Lead III of the electrocardiogram was observed in 14.4 per cent of patients not treated with digitalis, and in Lead I or in a combination of Leads I and II, or II and III, in 2.4 per cent of cases. T wave inversion in Lead III alone is known to be associated frequently with respiratory changes, but inversions in other leads are more probably related directly to the pneumonia.

Other changes in the form of the T wave and S-T segment were observed, but because of the lack of sufficient data with reference to the degree of variability of their form in the normal electrocardiogram, a detailed analysis has been avoided.

#### *Serum therapy and the electrocardiogram*

About one third of the cases studied in our series received intensive treatment with specific antipneumococcus serum. Thus, the possible influence of the administration of serum on the electrocardiogram was investigated. The incidence of abnormalities of rate, rhythm and conduction was found to be practically the same in the serum treated and untreated groups. Increased auriculoventricular conduction time during fever occurred somewhat more frequently in serum treated cases. However, there being a total of only eleven such cases, this fact cannot be made significant. It may therefore be stated generally that the serum administered does not influence the electrocardiographic findings.

#### SUMMARY

From the data we have presented it is evident that although electrocardiographic changes in lobar pneumonia are not so frequent as in certain other diseases, notably rheumatic fever and diphtheria, they occur often enough to be of significance. In approximately one quarter of all our cases, transitory changes were detected by the electrocardiogram at some time in the course of the disease. 10 per cent showed abnormalities of rhythm, 10 per cent showed disturbances of conduction, and in a small proportion, 24 per cent, inversions of the T wave other than in Lead III were noted.

A remarkable fact is that many of these changes occurred first in the postfebrile period. Marked sinus bradycardia, prolongation of auriculoventricular conduction time and intraventricular block occurring during convalescence have already been discussed. Also in two instances auricular flutter appeared after the temperature had begun to fall by lysis, and in the case reported by Shearer, the maximum degree of change in the R-T segment did not develop until the temperature had begun to fall. The evidence seems to indicate that whatever changes the pneumonic infection produces in the heart, these changes persist into, and may appear for the first time in the afebrile period.

## 650 ELECTROCARDIOGRAPHIC STUDY OF HEART IN LOBAR PNEUMONIA

While specific pathological changes in the heart have not yet been demonstrated in pneumonia, we do not feel that this militates against the interpretation of our findings. We do not attempt to place these observations on any definite structural basis.

### CONCLUSIONS

1. The heart rate is a valuable guide to prognosis throughout the course of lobar pneumonia. The expectancy of life diminishes as the heart rate increases, this correlation being even more pronounced in the later days of the disease.

2. The heart rate in pneumonia in a large proportion of cases remains fairly constant during the febrile period, with only slight variations from day to day and in many instances despite fluctuations of temperature.

3. Slow heart rates may occur in pneumonia even in the presence of high temperatures.

4. Abnormalities of rhythm occur in about 10 per cent of our cases.

5. Abnormal basic rhythms are found in about 5 per cent of cases, and in these the prognosis is poor.

6. The occurrence of premature contractions of ventricular origin does not alter the prognosis. Premature contractions of auricular or nodal origin may lessen the chances of recovery.

7. Conduction defects in lobar pneumonia occur in about 10 per cent of our patients not treated with digitalis.

8. In lobar pneumonia disease processes are active in the heart even after the temperature has become normal. Evidence to support this statement is found in the occurrence of auriculoventricular and intraventricular block and sinus bradycardia in the afebrile period of the disease.

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PROCEEDINGS OF THE TWENTY-THIRD ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N J, MAY 4, 1931

*The Effect of Phosphate Ingestion in Clinical Hyperparathyroidism with a Note as to What This Teaches Us Concerning the Physiology of the Parathyroid Glands* By FULLER ALBRIGHT and (by invitation) JESSIE REED COCKRILL, Boston Mass

Hyperparathyroidism is associated with four well recognized alterations in calcium and phosphorus metabolism (1) hypercalcemia (2) hypophosphatemia, (3) hypercalcinuria, and (4) hyperphosphaturia According to the theory of Albright and Ellsworth these are interrelated facts and dependent on a primary action of parathyroid hormone on phosphorus metabolism. They believe the administration of the hormone produces an increased urinary phosphorus excretion with a resulting decreased serum phosphorus level The calcium deviations from the normal are, they believe, secondary to and dependent on the phosphorus changes As a test of this theory phosphate by mouth was administered to patients with clinical hyperparathyroidism in the hope that this would raise the lowered serum phosphorus that the raising of the serum phosphorus would lower the serum calcium and that the lowering of the serum calcium would decrease the calcium excretion in the urine This was accomplished in two patients and the data of one of these patients are briefly presented Inasmuch as the metabolic abnormalities of hyperparathyroidism can be altered in the direction of normal by influencing the phosphorus metabolism, support is added to the theory that the parathyroid hormone produces these alterations in the first place by acting on the phosphorus metabolism

*Direct Measurements of the Oxygen Consumption of Isolated Beating Auricles from Normal and Thyrotoxic Guinea Pigs* By DONALD MCEACHERN (by invitation) and E COWLES ANDRUS, Baltimore Md

Last year a report was made before this society showing that the auricles and the hearts of thyrotoxic animals continued to beat when isolated, at a much faster rate than similar preparations from normal animals. This seemed to indicate a persistence of the specific thyroid effect on the isolated tissue. It seemed of interest therefore to study directly the oxygen consumption of these preparations

In a modified Warburg respirometer direct estimations were made of the oxygen consumption of isolated auricles from normal and thyrotoxic guinea pigs In a series of over 80 experiments the oxygen consumption of the

latter preparations was found to be greater than that of the normals. For a series of animals weighing between 10 and 20 mgm<sup>1</sup> the increase amounted to 7.3 per cent; for a series weighing between 21 and 30 mgm, 11 per cent; and for a series weighing between 31 and 40 mgm the increase in oxygen use amounted to 20.7 per cent above that of the normals.

These increases are considered greater than can be accounted for by the simple increase of rate of beat in the preparations from thyrotoxic animals. The increased oxygen use is thought to indicate an enhanced rate of metabolism in the isolated tissues due to the action of thyroxine. Experiments are under way to determine the same point in regard to resting tissues.

*The Effects of Pituitary Anti-Diuresis in Epilepsy.* By IRVING MCQUARRIE and (by invitation) DANIEL PEELER Minneapolis, Minn.

The relationship previously shown to exist between the state of hydration of the body and the occurrence of seizures in severely epileptic children has been found to hold also for early or mild cases under special conditions. Heretofore no satisfactory method has been available for the early diagnosis of the disease especially during the long free intervals between attacks. The present study has demonstrated, however, that convulsions or petit mal seizures can be induced almost at will in these patients by placing them on a special regimen with low mineral and relatively high water intake while administering the anti-diuretic principle from the hypophysis cerebri at sufficiently frequent intervals to prevent water diuresis. Seizures occurred regularly in a fairly large series of epileptic subjects when amounts of water equaling from two to four per cent of the body weight had been retained. Non-epileptic control subjects did not have seizures of any kind under the same or even more rigorous conditions.

That the convulsive reactions so produced are not due to the direct pressor action of the extract on the cerebral vessels nor to increased intracranial pressure *per se* has been fairly satisfactorily shown. Under the conditions of this special procedure the extracellular body fluids probably become sufficiently diluted to cause inhibition of a large excess of water by the brain cells. Preliminary data point to a resulting disturbance in the ionic equilibrium between the fluids on the two sides of the cell membrane. When an amount of NaCl calculated to be just sufficient to prevent this dilution was added to the diet seizures either did not occur at all or occurred only after a much greater gain in weight had been produced. The more or less specific response of epileptic patients to this procedure suggests that the inherent functional abnormality of the brain cells which is vaguely alluded to by various authors as characterizing the chronic convulsive state, may be brought to light by further investigations along this line.

<sup>1</sup> Dry weight.

*The Effect of Epileptic Convulsions and the Ketogenic Diet on Water Balance* By FRANK B BYROM (by invitation) and RUSSELL M. WILDER Chicago Ill

Water balance studies were made in three epileptic patients, using methods based on the work of Benedict and Newburgh

The individual convulsions of epilepsy usually provoke a loss of water (up to 1000 cc.) yet subsequent convulsions may occur within a few hours of such dehydration, and be absent in the reactionary period of water retention. These fluctuations coincide with the alterations in sodium excretion described by Gamble and Hamilton

A ketogenic diet induces an initial loss of 1000 to 2000 cc. of body water. A withdrawal of fixed base (sodium) accompanies it

Attacks may occur despite such dehydration. Coincident with the adequate mobilization of the urea ammonia mechanism for economizing fixed base the rate of water loss diminishes and equilibrium is reestablished at a lower level. A temporary negative nitrogen balance also occurs

These experiments indicate that when the body water is allowed to adjust itself in response to fluctuations in fixed base the onset of the convolution is not simply determined by the level of body fluid.

*Osmotic Pressure and Electrolyte Concentration of Blood Serum in Acute Alcoholic Intoxication and in the Acute Psychoses of Chronic Alcoholism* By ROY H TURNER New Orleans La.

Blood serums from seventeen individuals in various stages of acute alcoholic intoxication and seven patients suffering from acute psychoses of chronic alcoholism have been studied. Total osmotic pressure was determined by cryoscopy. The chemical determinations made were total fixed base, chlorides,  $\text{CO}_2$  content, albumin and globulin, inorganic phosphate, glucose, nonprotein nitrogen and alcohol. Osmotic pressure was calculated from the results of chemical analyses and calculated values were compared with determined values.

In the individuals with acute alcoholic intoxication osmotic pressures due to electrolytes were within the normal range no marked disturbances of acid base equilibrium were found. The total osmotic pressures were found to be increased roughly proportional to the degree of intoxication being above the physiologic range in all who were definitely intoxicated and reaching levels above 370 osmolar millimoles in comatose individuals. The increased osmotic pressure was due chiefly to alcohol in the serum.

In four patients showing acute psychosis of chronic alcoholism, total electrolyte concentration was less than normal though total osmotic pressure as determined was normal. In another such patient though total electrolyte concentration was normal base bound by organic acids was greatly increased and chloride concentration was greatly diminished. The serums of three other patients with mild brief symptoms showed little that was abnormal.

It is suggested that the increased osmotic pressure of the blood serum in acute alcoholic intoxication may play an important part in the disturbed physiology and in chronic alcoholism may bring about undue loss of body electrolyte which along with inadequate electrolyte intake may result in diminished electrolyte concentration in body fluids and cells and thereby play an important part in the production of delirium tremens and alcoholic hallucinosis. The relationship of these findings to various clinical features of alcoholism is discussed.

*Spinal Fluids in Hypertensive Disease* By JAMES P. O'HARF and (by invitation) SAMUEL A. SHELBYNE and DANIEL BLAIN Boston, Mass.

In an attempt to find out whether lumbar puncture might be used as a therapeutic agent in hypertensive conditions we submitted fifty patients with high blood pressure to this maneuver. The dynamics of the spinal fluid were investigated together with the blood pressure, the condition of the optic discs, X-rays of the skull and the renal function. The results of the studies may be summarized as follows:

Spinal fluid pressure was found to be increased in twenty out of fifty cases of hypertension, or forty per cent. The increased pressure occurred independently of renal function but was more often associated with renal insufficiency. Increased spinal fluid pressure was in every case associated with edema of the optic discs and higher spinal pressures seemed paralleled by greater choking. In every case but one increased spinal fluid pressure was associated with a diastolic blood pressure of 120 mm. Hg or more, although there were eight cases of diastolic blood pressure above 130 with a normal spinal fluid pressure. The average blood pressures in the cases with increased spinal fluid pressures were about twenty points higher in both the systolic and diastolic phases. X-rays of the skull showed increased pressure markings in only two out of thirty-two cases. These two were in patients who showed increased spinal fluid pressure. Headache was more frequent with increased spinal fluid pressure but occurred without it.

*Fowler's Solution in the Treatment of Chronic Myelogenous Leukemia* By CLAUDE E. FOWLER, T. F. McNAIR SCOTT (by invitation) and GEORGE R. MINOT, Boston, Mass.

Medical literature prior to 1903 contains numerous reports concerning the value of arsenic in chronic myelogenous leukemia. Distinctive data regarding the effect of this element are few. They indicate, however, that the administration of Fowler's solution may reduce the number of circulating white blood cells in leukemia and cause clinical improvement.

With the advent of roentgen ray treatment the administration of Fowler's solution fell into disrepute.

The effects of Fowler's solution have been studied in ten cases of chronic myelogenous leukemia. Nine of these cases have shown the following responses to treatment brought about in from three to five weeks:

- (a) The total number of white blood cells was reduced to near normal limits or even to below normal
- (b) The immature cells practically disappeared from the blood.
- (c) In every case the progress of the anemia was arrested and in most of the cases the red blood cell and hemoglobin values rose to near the normal
- (d) The blood platelets remained in normal or moderately increased numbers even in the leucopenic phases
- (e) The spleen and liver were reduced in size in all cases. In two cases, each with a large liver and a spleen extending to below the level of the umbilicus these organs were no longer palpable
- (f) The basal metabolic rate was reduced to normal
- (g) The patients gained weight and were subjectively and objectively improved
- (h) During the fall in the number of leucocytes the numbers of nucleated red blood corpuscles and the relative numbers of polymorphonuclear basophils and monocytes may be greatly increased
- (i) When Fowler's solution was omitted the signs and symptoms returned in a few weeks but the improvement could be maintained at least for a few months by continued small doses of the drug

One case of six years duration and in the terminal stage of the disease failed to respond to treatment.

These uncompleted observations suggest that Fowler's solution is of definite value in the palliative treatment of chronic myelogenous leukemia and perhaps can be used in conjunction with roentgen ray therapy for the best known sort of palliative treatment.

*The Relation of Available Iron to Acid and Alkaline Diets* By HERMAN H RIECKER, Ann Arbor, Mich.

Previous experiments upon dogs having chronic anemia from hemorrhage had suggested that in this type of anemia the sole underlying factor was iron starvation. This conclusion was refuted by Whipple who found an added effect upon blood regeneration when whole liver was added to inorganic iron. Kiefer reported similar results in chronic anemias of patients and Mettler and Minot found that hydrochloric acid administration with iron caused an increased response of the reticulocyte count, over that induced by iron alone.

The explanation for these diverse results is derived from the very sensitive solubility of iron salts in acid and is applied to cases having an anemia of iron starvation.

Experimental studies on this point were carried out as follows. In a normal individual the daily output of urinary iron was determined and its relation to diet to excessive iron intake and to excessive fluid intake.

In a patient recovering from pernicious anemia with achlorhydria (having large reserve iron stores) the urinary output of iron could be increased

more than 100 per cent by administering sodium bicarbonate. In the same patient the excretion decreased when small amounts of ammonium chloride were given. A diet neutral as to salts and a constant water intake were provided.

The experiment was repeated using a normal dog in which the total iron metabolism (food intake, hair, stool and urine output) was determined confirming the result that a highly acid salt ( $\text{NH}_4\text{Cl}$ ) held iron in the body.

Finally patients with severe secondary anemia from hemorrhage were used repeating the previous procedure to show that absorbed iron in iron starvation became increasingly available for blood formation.

It is believed that the results of Whipple on dogs and Kieler on patients using large amounts of whole liver are explained on the basis that liver being a highly acid food conserves the available iron in cases of iron starvation for utilization in blood formation.

Since 100 grams of liver have an acid equivalent of 10 cc of N/10 HCl, and an iron content of 83 mgm, its value in iron starvation is quite apparent.

*Mineral Metabolism during Treatment of a Case of Polycythemia Vera* By  
SAMUEL H. BASSETT (by invitation) and W. S. McCANN, Rochester,  
N.Y.

A case of polycythemia vera presenting the classical features of this condition namely brick red color of face, hands, and feet, enlargement of the spleen and polycythemia, was studied during a period of treatment with acetylphenylhydrazine. Blood studies and mineral balances, including nitrogen, phosphorus, calcium, magnesium, and iron, were carried out simultaneously. The administration of 2.9 grams of acetylphenylhydrazine in doses of 0.2 to 0.3 gram per day resulted in marked reduction of total blood volume, cell volume hemoglobin, and erythrocyte count. An increase in leucocyte count, icterus index and plasma volume occurred during blood destruction.

The destruction of approximately 900 grams of hemoglobin in 20 days with consequent liberation of about 3 grams of iron did not result in loss of body iron. Hemoglobinuria is believed to have accounted for increased iron excretion in the urine during the periods of erythrocyte disintegration.

Marked loss of nitrogen took place during the periods of blood destruction but the loss did not seem of sufficient magnitude to account for all the nitrogen contained in the destroyed erythrocytes.

Significant changes in the metabolism of phosphorus, calcium, and magnesium were not demonstrated.

*The Presence of Heterophilic Antibodies in Infectious Mononucleosis* By  
JOHN R. PAUL and (by invitation) W. W. BENNETT, New Haven,  
Conn.

Heterophilic antibodies are antibodies having the capacity to react with certain antigens which are quite different from and phylogenetically un-

related to the one instrumental in their production. Horse serum for instance when injected into human beings, may produce agglutinins for sheep cells and their presence as has been recently shown by Davidsohn, seems to parallel clinical features of serum disease.

That heterophilic antibodies demonstrable in the form of sheep cell agglutinins develop in cases of infectious mononucleosis in even higher concentration than in serum disease, is described in this report.

In our study of four cases, their presence was a constant feature of the acute phase of the disease while an excess of nononuclear cells was present in the blood. Among other common blood dyscrasias including lymphatic leukemia heterophilic antibodies were not demonstrated. They were noted however in a single obscure case thought to be aplastic anemia.

The reaction described in this disease must be essentially non specific, although it would seem to be of practical diagnostic value. Our interest centers mainly upon its occurrence in an infectious disease of obscure nature. Theories of the possible mechanisms involved are briefly discussed.

*The Significance of the Type Specific Skin Test in the Serum Treatment of Pneumonia (Type I)* By THOMAS FRANCIS, JR (by invitation), and WILLIAM S TILLETT, Baltimore Md

A study was made of the cutaneous reactions to the specific capsular polysaccharide of Type I pneumococcus in Type I pneumonia patients. A positive reaction which takes the form of an immediate wheal and erythema, is first elicited at or about the time of crisis and is always associated with the presence of circulating type specific antibodies. In patients treated with Type I antipneumococcus serum it was observed that the mere presence of circulating antibodies was not the only essential factor in the production of a positive test.

Of 35 cases studied 31 received serum. Four untreated control cases gave positive reactions at the time of recovery. In 5 fatal cases no positive reactions were obtained. Of the 26 serum treated patients who recovered 25 gave positive reactions when recovery began but not before. The one recovered case with negative skin tests developed empyema.

A positive reaction denotes recovery and serum therapy may be safely discontinued. A negative reaction is an indication for further administration of serum. If in the face of prolonged treatment, the reaction remains negative, a purulent complication should be suspected. If none is found serum administration should be continued although the prognosis is unfavorable.

*Experimental Lobar Pneumonia in the Dog* By E E TERRELL (by invitation) O H ROBERTSON and (by invitation) L T COGGESHALL, Chicago, Ill

Using a method previously described by us for the production of pneumococcus lobar pneumonia in the dog consisting essentially of the intra-

bronchial implantation of pneumococci suspended in a starch broth mixture, a study has been made of the evolution of the experimental disease from its inception to the complete disappearance of the lung lesions. The disease can be induced constantly with very small quantities of pneumococci; it resembles human lobar pneumonia in that the process produces complete consolidation of a lobe then spreads from lobe to lobe, is characteristically localized in the lungs and usually terminates abruptly. It differs from the typical disease in human beings by running a shorter course—usually three to five days, although it sometimes lasts six or seven days. One attack does not confer immunity against subsequent infection—although the succeeding attacks are milder in character, indicating the acquisition of immunity. No evidence of allergy to the pneumococcus was obtained either by skin reaction or intrapulmonary injection of the pneumococcus autolysate. The lungs show at different stages engorgement and red hepatization and if the disease lasts six to seven days a modified gray hepatization occurs. Studies of the pathology of the lesion reveal a histology in its main features analogous to the picture of lobar pneumonia in the human being.

*Natural Immunity of Man to the Pneumococcus* By W D SUTLiffe, MAXWELL FINLAND (by invitation) and HENRY JACKSON, JR., Boston, Mass

A search has been made by means of various immunological methods for measurable differences in susceptibility to pneumococci of Type I, Type II, and Type III among persons of different age groups.

Relatively few persons possess blood pneumococcidal power for Type I pneumococcus, nearly all possess blood pneumococcidal power for Type II pneumococcus, and an intermediate number possess blood pneumococcidal power for Type III pneumococcus. The blood of infants obtained at birth from the umbilical cord possesses pneumococcidal action fairly frequently. Samples of blood at the time of delivery from the mothers of these infants give the same results. In the group of infants aged from 1 month to 15 months pneumococcidal action is comparatively rare for all three types. In the age groups 2 to 11 years and 20 to 40 years pneumococcidal action is met with increasing frequency. In old age, fewer persons possess pneumococcidal power.

Protection tests and agglutination tests gave comparatively few positive results and thus do not afford reliable comparisons.

The skin reaction to the specific soluble substance could not be interpreted in the infants due to the usual appearance of a marked erythema lasting 30 minutes or more. No characteristic wheals were seen. In the other age groups the number of positive reactors to the specific soluble substance is fairly constant. Type II soluble substance gave rise to reactions more frequently than Type I or Type III soluble substances.

The percentage of reactors to the pneumococcus protein and autolysate is low in the lower in infants born in the other age groups. This is also true of the protein derived from the *Streptococcus hemolyticus*.

An incidental finding in connection with the skin tests is that of the appearance of antibodies following the skin tests

The fluctuation in the incidence of pneumococcal power with age resembles inversely the mortality curves of pneumonia with age. The frequency of whole blood pneumococcal power among the admissions to the Boston City Hospital is the reverse of the incidence of the three types in the pneumonia cases in the Boston City Hospital.

The reactions to intradermal injection of pneumococcus proteins and autolysates which are less frequent in infants than in any other age group indicate that individuals during childhood become sensitive to proteins contained in the pneumococcus as they do to other bacterial proteins.

*A Study of the Role of the Phagocytic Mononuclear Cell in Experimental Syphilis* By HUGH J MORGAN and (by invitation) SEALE HARRIS JR., R. S CUNNINGHAM and EDNA H TOMPKINS Nashville Tenn

Morgan<sup>1</sup> has shown that a basic feature of the microscopic pathology of experimental syphilis is an increase in size number and physiologic activity of phagocytic mononuclear cells of the tissues. The role played by these cells in the pathogenesis of syphilis is being studied.

There is evidence (especially in the work of Simpson<sup>2</sup>) that intravenous injections of trypan blue stimulate the production of phagocytic tissue mononuclear cells. On this assumption a series of experiments has been undertaken to investigate syphilis in animals receiving trypan blue.

In one experiment rabbits with well developed syphilitic lesions were given trypan blue intravenously. It was found that the lesions in these animals resolved more rapidly than in a control group receiving no dye. The dye did not sterilize the lesions of treponema. This was demonstrated by subsequent transference of the infection to normal rabbits.

In a second experiment trypan blue was given rabbits at the time of inoculation with *Treponema pallidum*. In these animals the lesions appeared later than in the control group which received no dye.

Further studies are being conducted in order to determine whether or not the modification of the disease observed in the above experiments is due specifically to the effect of the trypan blue on the mononuclear cells or to some other factor.

*Studies in Rheumatoid Arthritis* By M H DAWSON and (by invitation) R. H BOOTS, New York, N Y

(a) *Bacteriological investigations* Bacteriological investigations have been carried out on the blood, synovial fluid and subcutaneous nodules from a large series of cases of rheumatoid arthritis. In particular attempts were

<sup>1</sup> Morgan M J Trans Assoc Am Phys 1930 xlv 67

<sup>2</sup> Simpson M J Med Res 1921 lxiii 77

made to confirm the results recently reported by Cecil Nicholls and Stansby. The results of all bacteriological studies have been negative.

(b) *Subcutaneous nodules* The studies on subcutaneous nodules reported last year have been extended. These observations indicate that the subcutaneous nodules occurring in rheumatoid arthritis constitute a classical lesion of the disease. Studies on the histogenesis of the lesions show an intimate relationship between the subcutaneous nodules in this disease and those found in rheumatic fever.

(c) *Sedimentation rate of the erythrocytes* The determination of the sedimentation rate of the erythrocytes has been found to be of distinct clinical value. The results of approximately 1000 observations may be briefly stated as follows:

(1) The sedimentation rate serves as a useful guide in the differential diagnosis of rheumatoid and osteo-arthritis.

(2) In rheumatoid arthritis the sedimentation rate parallels to an extraordinary degree the activity of the process.

(b) *Agglutination reactions with hemolytic streptococci* It has been found that the sera of patients with rheumatoid arthritis possess the property of agglutinating hemolytic streptococci to an extraordinarily high titer. The observations indicate that the agglutination phenomenon is confined to hemolytic streptococci. The sera of a large series of control cases have failed to show evidence of agglutinins for the strains of hemolytic streptococci examined. The hypothesis is advanced that the disease rheumatoid arthritis results from infection with *Streptococcus hemolyticus*.

*The Formation of Glycogen in Mammalian Muscle from d-Lactic Acid and Glucose* By J. C. MEAKINS and (by invitation) C. N. H. LOON, Montreal.

The work of Neverhof and others has shown in the exercised isolated frog's muscle that as the preformed lactic acid disappears an equivalent of four-fifths of it reappears as glycogen. Furthermore Neverhof has found that perfusion of frog muscle with sodium lactate leads to the formation of muscle glycogen although others have been unable to confirm these results (Feggleton and Evans).

In the mammal the conversion of lactic acid into muscle glycogen either after exercise or during perfusion, has not been satisfactorily demonstrated.

In these experiments an attempt has been made to compare the glycogenic power of d-lactic acid with that of glucose and insulin. The decapitated eviscerated cat was used and the solutions slowly and continuously injected into a jugular vein by a Woodvatt pump. The d-lactic acid was used in the form of half-neutrallised 5 per cent or 10 per cent solutions.

The results obtained to date are as follows:

(1) d-lactic acid forms muscle glycogen in the cat in the absence of insulin, about 50 per cent less effective in this respect than are equivalent concentrations of glucose and insulin.

(2) A large proportion of the lactic acid disappearing is not found as muscle glycogen and in some experiments the oxygen consumption does not account for all the missing portion. This last conclusion is a temporary one only as we have not yet constructed an accurate balance sheet under these conditions.

We are indebted to Prof W H Peterson of the University of Wisconsin, for supplying us with pure d lactic acid.

*Calcium Metabolism in Calcinosi Universalis' or Calcification of the Subcutaneous Tissues* By WALTER BAUER and (by invitation) ALEXANDER MARBLE and GRANVILLE A BENNETT, Boston Mass

The occurrence of calcareous nodules in the subcutaneous tissues is a rare condition, affecting particularly children. Its etiology has remained obscure. It was hoped that a more detailed study of a boy of ten years of age, who had been known to have the disease for seven years might lead to a better understanding of this obscure condition. Therefore studies of calcium, phosphorus and nitrogen metabolism in conjunction with chemical and histological examinations of the biopsied material were made.

Chemical examination of the calcareous material showed it to be composed of calcium carbonate and calcium phosphate in proportion essentially the same as that of normal bone or pathological calcifications found elsewhere. There were no findings to suggest any gross abnormality of the uric acid, cholesterol or fat metabolism.

Histological study of the biopsied material revealed none of the abnormalities usually associated with pathological calcification namely chronic inflammation, hemorrhage, infarction, tissue injury or tissue necrosis. Examinations of the earliest lesions revealed a deposition of finely divided calcium particles around the periphery of the fat cells, which otherwise appeared to be normal. This process progressed until calcium had been deposited within fat cells and eventually replaced whole fat lobules.

Metabolic studies showed that the serum calcium and phosphorus values were normal. However, there existed an extraordinary ability to retain calcium and phosphorus. This tendency was more marked in the case of calcium than of phosphorus. Regardless of the calcium intake or the medication employed the fecal calcium excretion never exceeded 49 mgm per three-day period. The urinary calcium was also abnormally low. The low urinary calcium in the presence of a normal serum calcium served as further evidence of this unusual ability of the tissues to retain calcium. Results obtained during certain study periods indicate that when calcium is first retained it is not necessarily accompanied by phosphorus.

As a result of these studies we concluded that the calcium and phosphorus metabolism is abnormal in these rare cases of calcification of the subcutaneous tissues. The increased retention of these salts was evidently due to an unusual affinity of certain tissues for these elements. We are unable to state

what intracellular changes unrecognizable microscopically, precede the deposition in these regions.

An inadequate calcium diet in conjunction with ammonium chloride medication would seem to be the only rational therapy to be employed.

*A Study of the Electrolyte Metabolism in Diabetic Acidosis Induced in Human Subjects* By ROBERT F. LOEB, DANA W. ATCHLEY and (by invitation) DICKINSON W. RICHARDS JR., and ETHEL M. BENEDICT,  
New York N.Y.

We have studied the changes in water and electrolyte balance resulting from the abrupt withdrawal of insulin in three diabetic patients and also the steps taking place in recovery when insulin therapy is again instituted.

The fluid intake was maintained at a constant level throughout the experiment. The patients received and ate identical meals each day. Every fifth day when a fresh supply of food was bought a complete duplicate day's diet was analyzed for water, K, Ca, total fixed base, P, Cl and N. The diets and insulin dosage were adjusted so that the patients remained sugar-free, in nitrogen balance and at approximately the same weight during the control period of 10 to 16 days. Twenty-four hour urine specimens were analyzed for NH<sub>3</sub>, total fixed base, K, Ca (Na and Mg being determined by difference), Cl, P, inorganic SO<sub>4</sub>, total N, creatinine, glucose, ketone bodies, titratable acid and in one case for organic acids. The pH was also measured. Stools were analyzed in 5-day periods for K, Ca, total fixed base, Cl, P, and total N.

At the end of the control period insulin was withdrawn abruptly and withheld until definite results had been obtained or until therapy was urgently indicated. The recovery period was studied and then an after period in which the patients were again stabilized.

At various times in the course of the experiments blood samples were taken and electrolyte analyses were made.

The first patient was a comparatively mild diabetic receiving 55 units of insulin a day in the fore-period. Upon the withdrawal of insulin he remained sugar-free for 3 days and only on the 7th day without insulin did he finally excrete as much as 20 grams of glucose in twenty-four hours. In the second case, upon the withdrawal of insulin (80 units a day) there developed a glycosuria of 63 grams in the first twenty-four hours and 120-140 grams a day for the rest of the period. During this interval without insulin practically no ketosis developed. There appeared, however, immediate and striking changes in the water, electrolyte and nitrogen balances. In the third case, the insulin deprivation was associated with immediate and marked glycosuria and the development of progressive ketosis severe enough to require active therapy when the blood CO<sub>2</sub> reached 30 volumes per cent on the fourth day. In this patient even more marked but

qualitatively similar disturbances of water and electrolyte balances were encountered.

*Results* (1) The withdrawal of insulin without the development of appreciable glycosuria or ketosis had no apparent effect upon water and electrolyte balance (2) The withdrawal of insulin when followed by marked glycosuria either with or without ketosis resulted in definite and immediate disturbances in electrolyte and water balances as follows (a) An enormous loss of water, Na, K and Cl beginning in the first twenty four hours and associated with loss in body weight and nitrogen. These changes were maintained at an abnormally high level throughout the period of insulin deprivation. (b) Twenty-four to forty-eight hours after the peak of Na, K and Cl loss the excretion of NH<sub>3</sub> began to be augmented (c) In the case which developed acidosis the ammonia increase roughly paralleled the increase in ketone acids (3) Upon restoring insulin therapy and thus marking the beginning of recovery, a series of dramatic changes took place Among these changes were (a) The immediate and striking retention of K, Na, Cl and water, associated with an increase in body weight but without the establishment of a positive nitrogen balance. (b) The NH<sub>3</sub> excretion in both cases, i.e. with and without ketosis, remained at an augmented level during the first 4 days of the recovery period This abnormally great NH<sub>3</sub> excretion bore no quantitative relationship to the disappearance of ketones, the restoration of total base or the replacement of Cl The excess ammonia excretion did however, cease in both cases on the day when the K which had been lost with intracellular water, was completely restored

Finally we should like to point out that the major fluctuations in weight in our experiments parallel the loss and storage of electrolytes rather than of nitrogen While these changes are definitely associated with glycogen loss and restoration and while a causal relationship has been generally assumed no satisfactory explanation for such a mechanism has been advanced

*Surface Temperature and Radiation* By W S McCLELLAN and E F DU Bois New York, N Y

The study of patients with malaria in the Sage respiration calorimeter suggested that the radiation of heat is not necessarily proportional to the surface temperature of the body A series of observations has recently been made determining the heat lost by radiation and conduction and also the temperature changes of seventeen different spots on the skin Surface temperature measurements are made by means of a small button of Woods metal in which an insulated wire having a resistance of 100 ohms is embedded With proper precautions the readings on the Kohlrausch bridge become constant in about ten seconds

Experimental subjects have been studied naked and clothed in the respiration calorimeter and the heat production has been varied by muscular activity

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In some experiments more heat was radiated from or through the skin when it was cold than when it was warm. This suggests that the radiating surface of the body is not necessarily the skin surface but may be located at some level underneath the skin and that the radiation of heat is not proportional to the surface temperature of the body.

*A Safe Method of Producing Hyperthermia in the Treatment of Disease  
1 Preliminary Report* By F W BISHOP, C B HORTON (by invitation) and S L WARREN Rochester, N Y

Radio waves are passed by means of large electrodes through the trunk or the patient with resulting rapid and safe rise in his general body temperature to  $41.5^{\circ}$  C within approximately 2 hours. The electrodes are then removed. The patient is maintained at this temperature in a warm air chamber from 4 to 6 hours. The patient is then given a cold glucose and saline enema removed to a dry bed and allowed to cool off a process taking about  $1\frac{1}{2}$  to 2 hours. The patients are slightly weak but can be discharged the following day.  $42^{\circ}$  C for more than a few minutes is a dangerous temperature.

Fifty treatments have been given to 25 patients in the current year with one death. Studies of blood chemistry, electrocardiograms, blood pressure, temperature and other data on patients and animals show no changes below  $42^{\circ}$  C which might have a deleterious effect.

Seven of 14 deteriorating paretic patients have been paroled or discharged. All have been clinically improved. The gain in weight has been from 15 to 30 pounds. Striking serological changes have been produced in the spinal fluid without arsenical treatment. Transient herpetic lesions of the face develop in 60 per cent of the cases.

*The Presence of Digitalis in Edema Fluid and its Possible Clinical Significance* By GEORGE H MILLER (by invitation) and FRED M SMITH, Iowa City, Ia

Edema fluids from patients who have been under prolonged treatment for cardiac failure have been assayed for digitalis by the Hatcher cat method. Fluids aspirated from the pleural and peritoneal cavities were employed except in two instances. In the latter, the fluid was obtained from the extremities by means of Southeys tubes.

Repeated assays were carried out on each sample to minimize the influence of individual variations in the test animal. Sixty-five assays were done on samples from fifteen patients. In each instance the fluid showed a digitalis titer. The fluid from the patient with the highest titer showed an average of 0.9 cat unit per 100 cc. The samples from the patient with the lowest titer averaged 0.46 cat unit per 100 cc.

Fluids were employed from patients who had not been treated with digitalis controls. These did not give a digitalis titer. On the other

hand when a known amount of digitalis was added to the control fluids, the full amount was indicated in the assays.

Patients with cardiac failure and edema who respond to treatment frequently excrete two to three liters of the excess fluid through the kidneys in twenty-four hours. It has been repeatedly shown that digitalis is not readily excreted by the kidneys.

Under the above circumstances, it is therefore possible for the digitalis of edema fluid to become effective and induce poisoning. Certain clinical observations support this possibility.

*Diuretic Action of Digitalis* By E. LEROY KELLUM (by invitation) and  
NORMAN M. KEITH Rochester, Minn.

Since Withering's original communication on digitalis there are relatively few instances of carefully controlled and standardized studies of digitalis on both the healthy human subject and the cases of congestive cardiac failure. In spite of the vast amount of work on this drug and because of our experience of the not infrequent failure to produce satisfactory diuresis by digitalis therapy in cases of congestive heart failure with edema, the present work was attempted to determine the efficacy of digitalis as a diuretic.

The diuretic effect of digitalis was studied on three healthy human subjects and on a series of cases with congestive heart failure and considerable edema. To insure standard experimental conditions all of the subjects were given a measured fluid intake and a weighed and constant diet of known low salt and low water content but adequate in protein and total caloric value. After a preliminary control and rest period with no medication a standardized digitalis preparation was given to each subject in adequate amount. Careful daily estimations were made of the body weight, the pulse rate, blood pressure, twenty-four hour urinary output and the clinical symptoms. Determinations of chlorides, fixed base and nitrogen were made on the daily urinary output.

Despite the fact that all the subjects both the healthy and the cases of congestive failure with edema, showed characteristic electrocardiographic and clinical evidence of digitalis effect the diuretic effect of this drug was only slight in some of the subjects and was entirely absent in the remaining subjects. In none of the subjects was a diuretic effect of digitalis as marked as we have experienced where certain other diuretic substances have been employed. It is suggested that in the treatment of congestive heart failure with edema digitalis be employed for its beneficial cardiac effect and other diuretics also be employed for their superior diuretic action.

*A Comparative Study of the Diuretic Action of Euphyllin and Salyrgan*  
HENRY L. SCHMITZ (by invitation) and LOUIS LEITER Chicago, Ill.

Rehberg's method of using creatinine to calculate the amount of filtration occurring in the glomeruli and the amount of reabsorption taking place in

the tubules has been adopted to compare the diuretic action of euphyllin and salvagan in the dog.

In the experiments to date euphyllin has consistently increased the calculated amount of glomerular filtration whereas reabsorption has seldom been decreased significantly. The urine output following injection of euphyllin has only once exceeded 2 cc per minute. Diuresis has not occurred when the control urine volume exceeded 15 cc per minute even though the calculated rate of filtration was not greater than in those experiments in which diuresis resulted.

In the experiments with salvagan on the other hand, there has regularly been a decrease in the calculated percentage of fluid reabsorbed while a significant increase in the amount of filtration has occurred only once. The diuresis produced by salvagan has always been greater than 2 cc per minute and has also occurred when the control urine volume exceeded 15 cc per minute.

These experiments suggest that salvagan acts in the kidney by decreasing tubular reabsorption while euphyllin acts largely by increasing glomerular filtration. Work is being continued along these lines.

*Unusual Variations of the Roentgen Shadow of the Elongated Thoracic Aorta.* By HUGO RÖSLER, Vienna (by invitation), and PAUL D. WHITE, Boston, Mass.

Unusual undescribed variations from the familiar roentgen picture of the tortuous elongated thoracic aorta deserve attention because of their importance in differential diagnosis. We have encountered three instances. First was a man 60 years old with arteriosclerosis and slight hypertension. The heart size was within normal limits. The descending aorta crossed the midline to the right on its way downward, displaced the trachea forward and extended far into the right lung field before turning back behind the heart. This finding is to be differentiated from the picture produced by aneurysm of the ascending aorta.

Second, a man of 50 years with arteriosclerosis and hypertension, showed a moderately enlarged heart, calcification in the aortic arch and a sharp kink of the aorta just beyond the arch subdividing the vessel in its course and to be differentiated from coarctation of the aorta.

Third, a man of 61 years with arteriosclerosis, tabis dorsalis and slight hypertension showed slight enlargement of the heart, moderate dilatation and calcification of the aortic arch and a marked kink, without change in caliber in the lower third of the descending thoracic aorta.

In all three cases the oblique views proved to be of the greatest value.

*The Use of Ephedrine in Complete Heart Block with Adams-Stokes Syndrome.* By J. EDWIN WOOD JR., University, Va.

In many respects epinephrine and ephedrine show a similar action. With

this in mind and the added stimulus of three favorable case reports the following clinical studies have been made.

The first of these summarizes the clinical record of a male patient 51 years of age who developed a severe Adams Stokes syndrome shortly after coming under observation. In spite of increasing doses of barium chloride, five to six severe attacks and 50 to 75 transient seizures daily almost completely incapacitated the patient. Immediate relief followed the administration of ephedrine by mouth with the entire absence of long diastolic pauses in subsequent electrocardiograms. Under continued oral administration of ephedrine the patient has experienced relief for more than a year. The constant use of ephedrine has not produced nervousness, unpleasant symptoms, or unfavorable signs.

In this case a 72 mgm. dose of ephedrine by mouth produced a gradual increase in the ventricular rate from 20 to 30. This increased rate was still present at the end of six hours. The long diastolic pauses present (several times the ventricular rate dropped as low as six per minute before ephedrine was given) at the beginning of the experiment completely disappeared under the drug. After nearly a year ephedrine was administered to this same case of complete heart block after full atropinization. A mild but definite ephedrine response occurred.

This patient experienced a violent and alarming reaction in the form of a ventricular tachycardia rate 112, following the injection of one mgm. of epinephrine subcutaneously.

Two other cases of complete heart block showed a definite increase in ventricular rate following the ephedrine administration. In one instance of paroxysmal complete heart block no evidence could be obtained to indicate that ephedrine improved conduction.

Observations on three cases of complete heart block indicate that ephedrine increases the ventricular rate independently of the auricular rate or blood pressure changes.

*The Response of the Circulation to Insulin Hypoglycemia* By A. CARLTON ERNSTINE, MARK D. ALTSCHULE (by invitation) and HERRMAN L. BLUMGART Boston, Mass.

Several patients with diabetes mellitus and early myocardial failure were observed recently in whom, following the administration of insulin, the symptoms of congestive heart failure increased in severity. Similar clinical experiences have been recorded by other observers the situation being occasionally complicated by the development of coronary thrombosis or attacks of angina pectoris. It seemed of interest consequently to study the changes in the circulation following the administration of insulin.

The pulse rate, arterial blood pressure and minute volume output of the heart were measured in sixteen normal non-diabetic individuals of various ages both before and again during insulin hypoglycemia. In most of the

subjects the pulse rate and pulse pressure were increased during hypoglycemia the systolic blood pressure being elevated and the diastolic pressure lowered. In every subject the circulatory minute volume measured according to the acetylene method of Grollman was increased, although in a few subjects the increase was not beyond the limits of error. The increase in minute volume output following insulin averaged 31.4 per cent and varied in different individuals from 31 to 85.7 per cent. The percentage rise in the minute volume output of the heart was not proportional to the extent to which the blood sugar level was lowered.

These observations indicate that insulin hypoglycemia is attended by an increased minute volume output of the heart and an increased amount of cardiac work. In normal individuals this increased work is accomplished without difficulty. In elderly subjects with sclerosis of the coronary arteries and diminished myocardial reserve the increased burden imposed on the heart may result at times in the development of conspicuous signs and symptoms of myocardial failure. The results of the present investigation, therefore furnish a rational explanation for the signs of circulatory insufficiency which have been observed during insulin hypoglycemia. In patients with arteriosclerosis or with signs and symptoms of circulatory insufficiency, insulin should be administered with appropriate precautions such as the giving of smaller amounts at more frequent intervals.

*Studies of Edema* By F. H. LASHMET (by invitation) and L. H. NEWBURGH, Ann Arbor Mich.

For the treatment of edema of nephritic origin various types of procedure have been proposed. Superficially, each type is diametrically opposed to the other. Evidence will be presented to illustrate the following points:

- (1) Restriction of fluid intake has no influence in preventing or eliminating edema.
- (2) Acids and acid producing salts will cause diuresis.
- (3) A neutral type of diet instead of the high alkaline nephritic diet commonly used increases the effectiveness of acid therapy.

Other observers have reported the same results by the use of huge doses of alkali. Since both acid and alkali therapy produce diuresis it seems that the reaction of the medication has less to do with diuresis than the osmotic effects which these drugs produce. Experiments are under way to test this hypothesis.

*The Influence of Water Balance on Manifestations in Pulmonary Tuberculosis* By BURGESS GOKKO and (by invitation) EN SHU TAI and R. I. THIFFINATOR Philadelphia Pa.

The daily variations in urinary output and the occurrence of perspiration, expectoration were recorded in twenty-four tuberculous patients. These were correlated with cases of dry cough, hemoptysis, cat-

ness and elevation of temperature. Records of the extremes in humidity and temperature of each day were kept.

It was found on certain days that marked differences in the output of urine occurred. There was an apparent relationship between increased fluid loss through the skin and the exaggeration of symptoms. A rather typical course was as follows. With a difference of more than 10° to 20° F in the minimum and maximum temperature or a difference of more than 15 to 25 per cent in humidity there was increased perspiration (frequently one or two night sweats). Urination was decreased, usually expectoration also and 12 to 24 hours later an elevation of body temperature (beyond the usual level for the patient) occurred. This was followed by an exaggeration of the clinical symptoms.

It is suggested that dehydration may explain in part the periodicity of certain manifestations in tuberculosis. Whereas increased fluid intake with free urination is helpful in removing toxic products the loss of fluid through sweating is perhaps harmful. This may explain the fact that tuberculous patients with good nutrition and apparently greater fluid reserve suffer less frequently from exacerbations than individuals underweight and with low storage of fluid. It would appear that equitable climate in undernourished patients with progressive disease is desirable or a plan of management which provides an increased fluid intake restricted exertion and sedatives on days with rising temperature and humidity.

*The Utilization of Glucose Derivatives by Cancer Cells* By WILLIAM T SALTER, OVID O MEYER (by invitation) and JOSEPH C AUB Boston, Mass

In studying differences between the biochemical behavior of malignant tissue (mouse sarcoma number 180) and that of normal tissues the utilization of various derivatives of glucose (in which successive chemical groups were systematically substituted) was measured under anaerobic conditions by Warburg's method. Barring differential permeability of those substances through cell membranes (and similar complications), such compounds may be considered as substrates the chemical change in which gives quantitative evidence of the activity of intracellular enzymes.

The splitting of hexoses ("glycolysis") is peculiarly restricted to d-glucose itself the chief exception being mannose (as Warburg pointed out). Of various simple derivatives of glucose most failed to show acid formation. That the configuration of the oxygen ring is of prime importance is indicated by the fact that Pringsheim's glucose (hexylene-oxide ring) showed no glycolysis. Glucofuranose compounds (butylene-oxide ring) however, were encountered, which did yield acid under anaerobic conditions though not so readily as normal dextrose.

Although no anaerobic acid formation was encountered with pentoses, it was definite in the case of several 4-carbon and 3-carbon aliphatic carbo-

hydrates Acid production from these substances by rapidly growing tumor, however may be less active than by normal tissue

Acid production was also observed in the case of polysaccharides, particularly with disaccharides containing glucose

*Studies on Obesity: the Specific Dynamic Action of Food* By FRANK A. EVANS and (by invitation) J. V. STRANG, Pittsburgh, Pa

The total extra heat produced in response to a fixed meal was the same in the obese and in those of normal weight. The meal was made up of 40 grams of protein, 52 grams of carbohydrate and 26 grams of fat, which gave 610 calories. The observed extra heat production averaged 57 calories or 9 per cent of the total food calories.

*The Peripheral Circulation in Heart Disease* By SOVA WEISS and (by invitation) LAURENCE B. ELLIS, Boston, Mass

In order to gain further knowledge of the state of the peripheral circulation in various stages of circulatory failure due to heart disease, studies were made of the oxygen utilization in the forearm and leg, both in the horizontal and upright positions and before, and at intervals after a standard walking exercise. These findings were correlated with the lactic acid contents of arterial blood and blood from the femoral and antecubital veins obtained at the same time as well as with the basal metabolic rate, vital capacity, cardiac rate, arterial and venous blood pressures and the clinical manifestations of each of the patients studied. The results obtained furnish significant information regarding the blood supply to the great bulk of the body musculature particularly of the legs and may be considered an index of the state of the entire peripheral circulation. A total of 17 normal subjects and 50 patients with cardiovascular disease was studied.

At rest, in the horizontal position, there is a tendency for the oxygen utilization in the arms and legs to increase as the heart failure progresses, although in several instances of pronounced circulatory failure normal oxygen utilization was observed. The lactic acid contents in the arm and leg blood were above the normal limits only in the presence of marked congestive failure and then not always.

In the upright position, both in normal persons and in patients with severe congestive failure the oxygen utilization, particularly in the legs, increases more than would be expected from the theoretical rise in the metabolism. The lactic acid contents of arm and leg blood showed no, or very slight, elevation in this position.

Upon exercise the oxygen utilization is greater in patients with cardiac failure than in control's or compensated cardiaques. In pronounced circulatory failure as high as 16.5 volumes per cent oxygen utilization was obtained and the oxygen content of the femoral venous blood reached as low as 10 volume per cent. In both normals and cardiaques the return of oxygen utilization to

the resting level occurred promptly, within ten minutes. Following exercise, the average rise in the lactic acid in the femoral blood draining active muscle in normals, was to 30 mgni per cent, much higher than in the arm but the two approached each other in about ten minutes and the lactic acid fell to a resting level in about one hour. In patients with circulatory failure, there was a tendency for the lactic acid to rise somewhat higher and fall more slowly. This difference between normal persons and patients with circulatory failure was probably due to slower blood flow, relative tissue anoxemia, and possibly was influenced by the liver damage accompanying heart failure.

In this study no evidence was obtained that in congestive circulatory failure there is an increased cardiac output or a more rapid blood flow than normal through edematous tissues. On the other hand, a decreased cardiac output is not regularly found in the presence of circulatory failure and the degree of the circulatory failure cannot be estimated by any change in the volume flow of blood. No evidence of a primary disturbance in the lactic acid metabolism or resynthesis was obtained.

*The Differentiation of Peripheral Arterial Spasm and Occlusion in Ambulatory Patients* By W J MERLE SCOTT and JOHN J MORTON, Rochester N Y

To differentiate the effects of spasm from those of occlusion is an essential step in the study of arterial diseases in the extremities. This can not be done by the usual methods of clinical investigation. The reaction to foreign protein and the effects of spinal or general anesthesia have previously been used to accomplish this differentiation. A satisfactory test to measure these elements is outlined. It consists in following the surface temperature in the distal parts of the extremities before and after the blocking of the nerve trunk to the area. The vasodilatation obtained in this way compared with that following spinal or general anesthesia is nearly as complete. This test is so simple that it is applicable without inconvenience to ambulatory patients. Examples of its application and value in Raynaud's disease, thromboangiitis obliterans and arteriosclerotic endarteritis are given.

*Cardiac Dyspnea. The Relative Importance of the Chemical and of the Reflex Control of Respiration in the Mechanism of its Production* By T R HARRISON and (by invitation) GLENN E. CULLEN, J A CALHOUN, W E WILKINS and CORN PILCHER, Nashville, Tenn

The mechanism of orthopnea and that of dyspnea produced by slight exertion have been studied in a group of patients with various types of cardiac disease. In an orthopneic subject the shift from the recumbent to the sitting posture was not usually accompanied by significant changes in the carbon dioxide content carbon dioxide tension pH or oxygen content of the arterial blood or of the blood from the internal jugular vein. The difference between the oxygen content of blood from these two vessels was the same as

that found in normal subjects and was unaltered by change of posture. Therefore orthopnea is probably not due to decreased cerebral blood flow. Increased vital capacity, decreased ventilation and decreased respiratory rate were usually observed in the sitting as compared to the recumbent posture.

Observations made on dogs showed that, when the vagus nerves were intact, artificial diminution in vital capacity produced by pneumothorax, by introducing Ringer's solution into the lungs or by distending the vessels of one of the lungs with blood caused increased respiratory rate and increased ventilation. No significant changes in the pH or the gases of the arterial blood or of the venous blood from the brain were observed until the decrease in vital capacity was of marked degree, whereas relatively slight decrease in vital capacity was accompanied by increase in respiratory rate and ventilation. After bilateral vagotomy reduction of vital capacity did not change the breathing until decreased oxygen content or increased acidity of the blood occurred.

It therefore appears probable that orthopnea and cardiac dyspnea at rest (aside from Cheyne-Stokes respiration and "cardiac asthma") are due to alterations in the Hering-Brauer vagal reflex from the lungs.

Dyspnea produced by mild exertion was also found to be unassociated with significant changes in the pH, carbon dioxide content or tension, or oxygen content of the venous blood from the arm, the blood from the internal jugular vein or the arterial blood. It is evident that this type of dyspnea is also of reflex nature but further studies are necessary in order to determine its nature more precisely.

Similar findings have been obtained on normal subjects and again with mild exercise chemical alterations in the blood were absent. It seems probable that the nervous regulatory mechanism is more delicate than the chemical and that the former is largely responsible for the changes in ventilation which occur with the usual activities of life aside from severe exertion.

*The Clinical Value of the Erythrocyte Sedimentation Test* By C. P. HOWARD and (by invitation) E. S. MILLS, Montreal, Canada

The corrected sedimentation velocity of the erythrocytes has been determined on 250 patients. Many of these patients were suffering from an acute or chronic inflammatory disease. Where possible the test was repeated at regular intervals during the course of the disease.

This test was compared with the leucocyte count, the pulse rate and the temperature at the time of the test in order to determine if possible the relative sensitivity of these factors in recording the degree of activity of the inflammatory process.

As a general rule it was found that the corrected sedimentation velocity is a better indication of activity in such inflammatory diseases as tuberculosis, dactylitis and the various forms of arthritis than is one or all of

the other factors with which the test was compared. The test is therefore a valuable aid in both diagnosis and prognosis in these diseases.

*An Analysis of Thyroid Clinic Data by a Code and Punch Card System*  
By J. LERMAN (by invitation) and J. H. MEANS Boston, Mass.

Methods for accumulating and analyzing clinical data in vogue at the present time are cumbersome and inadequate. The application to clinical statistical problems of mechanical accounting systems such as are used in business was first proposed by Raymond Pearl. Believing these methods worth further study, we have applied them to the material of a Thyroid Clinic. The hope is that should we be able to demonstrate their value in a special clinic it might pave the way to application of the methods to all hospital entries.

A special coded record for thyroid cases is presented. The method of transfer of its information to statistical punch cards and the classification of these by a sorting machine are described. The results of the past year have been analyzed. Examples are given to illustrate significant findings and correlations for conditions such as exophthalmic goiter, colloid goiter, nodular goiter and myxedema.

A great many questions which are constantly being asked can now be answered with specific facts and figures rather than by impressions.

*Age and the Susceptibility of Rabbits to Scarlet Fever Toxin* By JAMES D. TRASK, New Haven, Conn.

The report of Parish and Okell on the greater susceptibility of mature as compared to young rabbits to lethal doses of scarlet fever toxin was confirmed and extended. It was found that old rabbits were more susceptible than young ones to skin test as well as to lethal doses of scarlet fever toxin, that with both methods the toxin was neutralizable with scarlet fever antitoxin and that the activity of the toxin was destroyed by heating to 100° C for two hours. It was shown that scarlet fever antitoxin was not present in detectable amounts in the blood of normal rabbits either young or old.

The above findings furnish data for a consideration of the natural acquisition of susceptibility to a bacterial toxin.

*Respiratory Responses to Anoxemia* By A. V. BOCK and (by invitation) D. B. DILL and H. T. EDWARDS Boston, Mass.

This study shows a wide variation in the reaction of ten normal men while breathing oxygen poor mixtures. The total ventilation, alveolar CO<sub>2</sub> pressure, arterial saturation with oxygen, pH and the partial pressures of oxygen and carbon dioxide in the blood were determined. The mixture breathed contained 9 per cent of oxygen.

Subsequent experiments on the same subjects with mixtures containing varying amounts of CO and O<sub>2</sub> (now in process) are leading to interesting implications.

The experiments from a theoretical point of view bear out the hypothesis that there is no single hormone or mechanism responsible for the control of breathing.

*Studies in Intestinal Anaphylaxis* By ALBERT J. SULLIVAN (by invitation)  
and FRANCIS G. BLAKE, New Haven, Conn.

In an effort to obtain some knowledge concerning the so-called intestinal anaphylaxis of man, a study of the enteral sensitization and intoxication of guinea pigs with normal human serum has been undertaken. Mature pigs of 350 to 500 grams were sensitized by the oral administration of 2 cc of human serum daily for ten days. No reactions were noted during this period. After an 18-day rest period the animals were subjected to the intravenous injection of 1 cc of the same serum. Fifty per cent died within 5 minutes, 25 per cent developed severe shock but recovered. In the remaining 25 per cent no reaction occurred.

In repeating the experiment with young pigs of from 110 to 250 grams it was found that severe reactions, consisting of vomiting, choking, convulsions and death frequently developed during or a few minutes after the oral administration of the serum. Control animals given water reacted likewise. It was therefore decided that because of this danger of aspiration, any further studies on small pigs must be conducted by the administration of the antigen through a stomach tube. Such studies indicate (1) that occasional mild and moderate reactions which may be anaphylactic in nature can be obtained by the stomachal administration of antigen to previously sensitized animals and (2) that animals sensitized intravenously are not easily desensitized by the stomachal administration of the antigen.

*Studies in Sleep* By H. G. WOLFF and (by invitation) W. HORSLEY GANTT,  
New York, N. Y.

The conditioned reflex was used as indicator, along with other reactions, in an attempt to determine the nature of sleep and what circumstances precede, occur during and finally dissipate it. Dogs were used as experimental animals and they were observed in an environment in which essentially all external stimuli could be controlled by the experimenter.

It has been shown that when a conditioned stimulus which has always been followed by food is no longer followed by its unconditioned stimulus (food) the conditioned salivary reflex will disappear or be extinguished. In our animals extinguishing well established conditioned reflexes was followed by marked alterations in the behavior of the animals. The salivary reflex diminished and ultimately became zero, the general motor activity of the animal decreased, the breathing was slowed and deepened, the eyelids drooped or closed, the animal rested or leaned against the apparatus and "acted" like every ordinary evidence of being asleep. Such experiments

tally induced sleep could be dispelled immediately and completely by once more accompanying the conditioned stimulus by its unconditioned stimulus

It was also observed that the act of establishing a difficult discrimination between allied stimuli was often followed by the same evidence of decreased motility eyelid and breathing changes and eventually sleep

These observations indicate that the disturbance created in the central nervous system under the described experimental circumstances becomes widespread in its influence They confirm those earlier reported by Pavlov and are most readily interpreted in terms of his concept It is found that sleep follows periods of internal inhibition i.e. negative excitation. In certain instances it is initiated by such a process involving first the cerebral cortex but subsequently including subcortical structures and their functions

*Reduction in the Total Number of Eosinophils in the Circulating Blood by Various Experimental Procedures* By A. IZARD JOSEPH (by invitation) and JOHN S. LAWRENCE Rochester N. Y.

Guinea pigs deprived of food for six to seven days almost invariably have shown diminished numbers of eosinophils in the circulating blood. A much more sudden and marked drop in the number of the eosinophils has occurred following the intraperitoneal injection of sodium bicarbonate, ammonium chloride and *B. coli*. The maximum drop with these latter procedures has been within six to twelve hours. It has been associated with a corresponding diminution in the number of lymphocytes in the peripheral blood. A similar response on the part of eosinophils has been shown to occur following the intravenous injection of sodium bicarbonate in the guinea pig, in the dog, and in one adult patient with trichinosis. Studies of sections from various organs of the guinea pig have indicated that the reduction in the number of eosinophils was not limited to the blood but involved the tissues (with the exception of the bone marrow). Finally it has been shown that the eosinophils can, within certain limitations perform some of the functions of the neutrophile in inflammation

*The Hemogram (Leucocytic Picture) in Convalescence from Acute Infectious Diseases* By PAUL REZNICKOFF New York, N. Y.

It is relatively easy to determine that a patient is acutely ill from an infectious disease but more difficult to decide when he is well. One of the best aids in gauging the convalescence of a patient is the hemogram or leucocytic picture of the blood.

Daily leucocyte and differential counts were made on 68 patients suffering from various acute infections. In the cases of 50 other patients counts were made at critical points in their illness.

In conditions such as pneumonia, tonsillitis and acute rheumatic fever recovery is presaged by a transitory monocyte increase. This occurs as a rule while many immature polymorphonuclear cells are still present. With

a return of the monocytes to normal and the presence of a normal number of immature polymorphonuclear cells the lymphocytes rise and remain elevated for a long time (35 to 50 per cent). An eosinophilic increase occurs with the lymphocytic phase. In acute rheumatic fever and tonsillitis and a few pneumonic patients an eosinophilic increase is seen early as well as late in convalescence.

In fatal cases the monocytes rarely increase and the lymphocytes are always depressed. A marked delay in lymphocytic and eosinophilic increase is characteristic of complications such as pleurisy in the course of pneumonia.

*Rheumatic Fever in Adult Porto Rican Immigrants* By E. P. BOAS, New York, N. Y.

Rheumatic fever and rheumatic heart disease are very rare in Porto Rico. Rheumatic infection is not uncommon among Porto Ricans who have immigrated to New York City. When infection occurs it is apt to be severe, and in adults tends to approach the childhood type of the disease. This occurred in 9 of 13 cases of active rheumatic fever in Porto Ricans studied at Mt. Sinai Hospital. A similar behavior of the disease has been noted in negroes who have recently come from the Southern States. The average age of the Porto Ricans at the time of their immigration was 19.8 years and the average age on the occasion of their first rheumatic infection 22.6 years. Severity of infection among the Porto Ricans was manifested by a stormy onset with chills and abdominal symptoms by the frequent development of pericarditis by a prolonged course and by higher mortality. The cause of the great severity of rheumatic fever in Porto Ricans living in New York remains obscure. It may be that they lack immunity due to the absence of subclinical infection in Porto Rico before their arrival in the United States. Again climatic factors may be determining. These data point to the importance of a more systematic and intensive study of the epidemiology and the prevalence of rheumatic fever in different parts of the world.

*Blood and Organ Changes in Rabbits Following the Intravenous Injection of Non-pathogenic Bacteria* By ROBERT N. NYE and (by invitation) VIDA F. RANDOLPH Boston, Mass.

Heavy suspensions of *B. subtilis* were injected intravenously in rabbits. Within a few minutes the circulating leucocytes, particularly the polymorphonuclear cells were markedly decreased. This change also occurred in "immune" and splenectomized rabbits, but the return to within normal limits was more rapid with the former. The majority of bacteria were removed from the blood stream within a few minutes in all animals.

In the normal uninjected rabbit there were about as many polymorphonuclear leucocytes in the liver as there were in all the circulating blood and there were about twice as many in the lungs. Following injection there

was a marked increase of these cells in the lungs and liver with a decrease of such cells in the bone marrow. The increase in the liver persisted for longer than that in the lung. The organ changes in splenectomized animals were similar. In "immune" animals the increases in the lungs and liver were more pronounced, but there was no decrease in the bone marrow. Large numbers of the bacilli were found in the lungs and liver, most marked in the former relatively soon after injection and in the latter several hours after injection.

On the basis of these observations certain facts relating to resistance in the rabbit can be obtained.

*Experimental Study of the Probable Chemical Basis Producing Systemic Reactions in Certain Cases of Cold Allergy* By GEORGE E BROWN and (by invitation) BAYARD T HORTON, Rochester Minn

Local and systemic histamine like reactions in cases of cold allergy have been observed in a series of nine cases. The local effects on the hands or feet consist of pallor, followed by redness, swelling and increased heat. After a latent period of three to four minutes a characteristic systemic reaction appears. This consists of a fall in the blood pressure, a sharp rise in the pulse rate, flushing of the face and a tendency to syncope with recovery in fifteen to thirty minutes. The local reaction persists for from eight to eighteen hours. The reaction suggests that which is observed after the injection of small doses of histamine. Further studies on the gastric secretion and electrocardiographic changes during the attack give further confirmatory evidence of the probable chemical basis of this unusual clinical syndrome. The release of histamine or histamine like substances from the skin as the result of cold seems probable.

*Oxygen Treatment in Chronic Pulmonary Disease* By ALVAN L BARACH and (by invitation) DICKINSON W RICHARDS JR., New York, N Y

Seven cases of chronic pulmonary disease have been treated over long periods (2 to 7 months) with high oxygen.

*Group I* Two cases had active pulmonary tuberculosis without cyanosis or signs of pulmonary insufficiency. No effect was noted on the patients general condition or on the course of the disease.

*Group II (a)* Two cases had active chronic pulmonary tuberculosis with extensive lung involvement, moderate cyanosis and dyspnea. They were treated in an oxygen chamber and (in one case) later by nasal catheter for 2 months. There was definite though only moderate improvement in general condition and symptoms. The course of the disease was not apparently altered.

*(b)* Three cases had chronic pulmonary fibrosis, non tuberculous with profound cyanosis and dyspnea. They were treated with high oxygen (cham-

ber or catheter) for 6 to 7 months. Marked improvement resulted. Two patients were restored to limited ambulatory activity.

Studies of arterial blood were made on one patient of Group II (*a*) and two of Group II (*b*). In one case the CO<sub>2</sub> increased by 34.5 volumes per cent in a second by 18.6 volumes per cent while in the third, after 7 months in 50 per cent oxygen, the arterial whole blood CO<sub>2</sub> content was 130 volumes per cent.

We have considered this rise in CO<sub>2</sub> to be in effect an adaptive change with high alveolar CO<sub>2</sub> the excretion of this gas by the lungs can be accomplished in spite of severely limited pulmonary ventilation.

*The Permeability of Blood Capillaries to Protein* By CECIL K. DRINKER  
and (by invitation) MADELINE TIFLD Boston, Mass

Since the work of Starling in 1894 it has been held that impermeability of capillaries to the blood proteins, except for the capillaries of the liver, spleen and intestine, is a general and fundamental property. Protein impermeability has been deemed essential in order to govern the movements of water out of and into the capillaries. It is considered proved by the fact that the glomerular choroidal and ciliary capillaries do not permit the passage of protein, and by the direct observations of Landis.

Experiments carried out during the past two years have convinced us that permeability of capillaries to the blood proteins is a more general property than impermeability. Lymph collected from many different sources in the same animal contains from 0.4 to 4.5 per cent of protein. This protein can be shown to consist of serum albumin, serum globulin and fibrinogen. It must come from the blood, since there is no evidence that the tissues in general or the lymphatic endothelium, can form it. The impermeability of renal choroidal and ciliary capillaries to protein is not evidence applicable to capillaries in general, since in all three of these cases the capillary membrane is reinforced by a second layer of cells. Our experiments indicate that lymph and tissue fluid are identical in composition and that the protein content of the tissue fluid is of prime importance in maintaining the water balance of the body.

*The Mean Electrical Axis in Bundle Branch Block and its Significance* By  
A. GARRARD MACLEOD, PAUL S. BARKER (by invitation) and FRANK N.  
WILSON Ann Arbor, Mich

If the electrocardiogram is enlarged by projection the area of the various deflections may be measured with a suitable planimeter.

From the areas of the initial deflections in the three leads the mean electrical axis during the QRS-interval may be determined by means of the principles of Einthoven's equilateral triangle.

The mean electrical axis determined in this manner gives the average direction in which the excitation wave spreads over the ventricular muscle.

The mean electrical axis in bundle branch block points from the contralateral toward the homolateral ventricle. Consequently the position of this axis is in accord with the view that the current interpretation of the electrocardiograms ascribed to human branch block must be revised.

In fact, a general law may be formulated that in a mass of excitable tissue the mean electrical axis will always point from the point or center of stimulation toward a determinable point in the tissue which in symmetrical masses coincides with the center of mass.

*Growth in Private School Children* By H GRAY and (by invitation) J G AYRES, San Francisco, Calif

For the study of growth, nutrition and diseases of the ductless glands accurate standards of build are necessary. Such standards exist only for a few characteristics for public school children in this country, and hardly at all for private school children. Since the latter differ in build from the former new data are especially desirable. The constitution of these children whose parents are characterized by professional or economic success, and who therefore by heredity and nurture may be expected to be future leaders is therefore here studied on the basis of fifteen physical measurements on about three thousand boys and about fifteen hundred girls from the ages of one to nineteen years. The topics treated are averages at each age and the variations around the average also differences between these children and those reported by others differences in racial stocks, types of build and the like. The results are designed to be of service to physicians concerned with questions of normal and abnormal development in this special category.

*Further Evidence as to Metabolic Disturbances in Bright's Disease* By G P GRABFIELD, Boston, Mass

It has been shown that the urinary sulphur and nitrogen excretion and the nitrogen sulphur ratio in Bright's disease is of a different type in patients with and without renal edema. The changes are of such a character as to make it probable that there is a fundamental disturbance in the intermediary sulphur metabolism in this condition.

It seemed desirable to extend these observations by observing the effects of salicylates and iodides on the nitrogen and sulphur excretion in Bright's disease. This has been done. All the subjects were on weighed diets and the figures are derived from three-day periods of observation. The drugs were administered for three days after meals the iodides in doses of 0.3 gram the salicylates in doses of 1 gram. It has been shown that both these drugs caused an increase of about 10 per cent in the nitrogen excretion in the urine of normal individuals but that the increase in nitrogen excretion after iodide is not accompanied by an increase in sulphur excretion whereas in the case of salicylates the two are parallel.

In studying patients with Bright's disease which have been reported elsewhere we have found that such patients without renal edema showed after sodium iodide an increase in the nitrogen-sulphur ratio, similar to, though to a lesser extent than that seen in the normal. The patients with renal edema showed the opposite reaction. It seems not unlikely that the patients exhibiting the nephrosis syndrome had some difficulty in the mobilization of deposit nitrogen by means of iodides.

When we consider the effect of sodium salicylate, we note that patients with nephritis show a similar reaction to that observed with iodide namely that the increase in the nitrogen excretion exceeds the increase in sulphur excretion. This is not due to a difficulty in the excretion of sulphur through the kidney, because it has been shown that these patients remain for long periods in negative sulphur balance and it is known that sulphur is more easily excreted by the kidney than nitrogen. Patients exhibiting the nephrosis syndrome showed a normal reaction to salicylates, so that in the case of this drug, as well as that of iodides, we have evidences of deviations from the usual reaction. These reactions are presented as further evidence of anomalies in the nitrogen and sulphur metabolism occurring in Bright's disease.

*The Prognostic Value of Blood Fat Curves after Adrenalin in Liver Disease* By CHESTER M. LOVES and (by invitation) JOSEPHINE C. WOOD  
Boston, Mass.

Determinations of the blood fatty acids following the injection of adrenalin have been made on thirty-five patients with various types of liver disease and in addition on a group of normal individuals and in certain disease entities in which there is a known disturbance of fat metabolism. Normally there is a sharp rise in the fatty acid during the first half hour after adrenalin followed by a more gradual fall to the previous level. Where the liver is diffusely damaged by an infiltrative process or by serious infection, we have noted either a lack of rise in the blood fat after adrenalin or an actual fall. Those cases that were clinically the sickest showed the greatest variation from the normal curve. In patients with extreme jaundice but with a good prognosis the shape of the curve was normal. The actual level of blood fat varied between extremely high and abnormally low figures. Patients with high blood fat and no liver disease failed to show the type of curves described above. We believe that is a test of liver function the above method has distinct prognostic value.

*Studies on Hunger, Insulin and Gastric Activity* By WALTER L. PALMER  
and (by invitation) THEODORE E. HEINZ New York N.Y.

In 1916 Carlson published his classical treatise on hunger in which he criticizes the earlier theories and the relationship between hunger and appetite,

and showed that the primary and essential factor in the hunger complex is the gastric sensation arising from contractions of the empty stomach. In 1929 Quigley, Johnson and Solomon reported that the subcutaneous injection of insulin produces an increase in gastric motility and tone and a prolongation of the hunger period. They concluded that insulin sensations, especially hunger parallel rather closely the degree of gastric activity.

The present work represents a study of the relationship between hunger, insulin reactions, blood sugar levels and gastric activity in 28 experiments on 17 subjects. The findings of previous workers were only partially confirmed. "Hunger pangs" were present in only four subjects. They were definitely coincident with gastric contractions, but were not increased in frequency or severity by insulin. The sensation of hunger, however, usually accompanied the insulin reaction and was fairly well correlated with the blood sugar level. Gastric activity was not clearly correlated with hunger, the blood sugar level or the severity of the insulin reaction. The results suggest that the extra gastric factors may be more significant in the genesis of hunger than is recognized at present.

*Studies of Acid Base and Water Balance in Edema* By A. B. HASTINGS and S. H. LIU (by invitation) and F. R. DIEUAIDE, Chicago Ill and Peiping, China

Measurements of the acid base and water balance have been made on two patients with nutritional edema and one with nephrosis over a period of approximately two months. These patients had total plasma protein values between 4.0 and 6.0 per cent and plasma albumin values between 2.0 and 2.8 per cent. All showed slight pitting edema. During the period of observation the acid base balance was displaced for intervals of four or eight days to the alkaline side by the administration of sodium bicarbonate and to the acid side by ammonium chloride or hydrochloric acid. The extent of the acid base displacement to the alkaline side was from serum pH 7.40 to 7.55 and from serum bicarbonate 26 to 40 millimols per liter; the displacement to the acid side was from serum pH 7.40 to 7.20 and from serum bicarbonate 26 to 14 millimols per liter. The effects of these acid base shifts may be summarized as follows:

(1) During the periods of alkali administration there occurred for the first two to five days an accumulation of water. Toward the end of the periods, a loss of water occurred in spite of the continued alkali administration. Upon the discontinuation of the alkali therapy the loss of water was prompt with a return to the original water balance level within four days. The amount of water accumulated during bicarbonate administration varied between 220 and 900 cc. The pitting edema usually became more noticeable while water was being retained. The plasma proteins while sometimes tending to decrease slightly during alkali ingestion did not vary extensively nor consistently.

In studying patients with Bright's disease which have been reported elsewhere we have found that such patients without renal edema showed after sodium iodide an increase in the nitrogen-sulphur ratio, similar to though to a lesser extent than that seen in the normal. The patients with renal edema showed the opposite reaction. It seems not unlikely that the patients exhibiting the nephrosis syndrome had some difficulty in the mobilization of deposit nitrogen by means of iodides.

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*Studies on the Mechanism of So called 'Opening Snap' (Claquement D'Ouverture de la Mitral) of Mitral Stenosis* By ALEXANDER MAR-GOLIES (by invitation) and CHARLES C WOLFERTH, Philadelphia Pa.

The generally accepted hypothesis that the characteristic diastolic snap or click of mitral stenosis is due to opening of the stenosed mitral valve has been subjected to analysis. Sixty cases of mitral stenosis as well as many normals and various types of cardiovascular disease were studied and the following data obtained. The sound was elicited only in mitral stenosis, the incidence being 60 per cent. It is a short snap or click heard best in the third or fourth left interspace. It begins 0.07 to 0.13 second after the second sound and if the latter becomes reduplicated may maintain its time relation to either component. The interval shortens as the cardiac rate increases. In auricular fibrillation it varies according to the length of the preceding ventricular diastole. The snap precedes the diastolic murmur by 0.03 to 0.05 second and the peak of the protodiastolic wave of the apex cardiogram by approximately 0.02 to 0.04 second. Comparison with roentgen kymograms of the left border demonstrates that the sound occurs at approximately the instant passive rotation has been completed.

The findings indicate that the characteristics of the 'opening snap' are different from those of any other sound heard over the heart and are in accord with the view that it is due to the vibrations produced in a stenosed valve when its opening movement in response to the pressure of blood from above is suddenly limited.

*The Cerebral Circulation XIV Changes in the Human Retinal Circulation and Cerebrospinal Fluid Pressure during Carbon Dioxide Oxygen Inhalation* By STANLEY COBB and FRANK FREMONT-SMITH Boston, Mass

Wolff and Lennox (Arch Neurol and Psych 1930 xxiii 1097) showed in animal experiments that breathing a mixture of carbon dioxide (90 per cent) and oxygen (10 per cent) caused a dilation of the cerebral vessels and an increase in the rate of blood flow through the brain so that the venous blood became more arterial. Coincidentally the cerebrospinal fluid pressure was increased. We have made analogous observations on man although our observations on the cerebral circulation are confined to the retinal vessels. Rebreathing a mixture of carbon dioxide (10 per cent) and oxygen (90 per cent) caused the retinal veins to become bright red and almost indistinguishable from the arteries the maximum effect being reached in about six minutes. The intracranial pressure was definitely increased during such breathing. It is probable that the cerebral vessels particularly the arterioles, are thus dilated in man as well as in animals and that the increased intracranial pressure is due to the cerebral vascular dilatation. It is interesting

(2) During the periods of acid administration essentially the opposite chain of events was observed. There was an immediate loss of water for two to four days followed by a gain which was especially marked after the acid was discontinued. With ammonium chloride the water loss amounted to 400 to 650 cc. During the periods of water loss a decrease of the pitting edema was usually recorded. Again the variations in plasma proteins were slight and rather irregular.

*Epidemic Seasonal Edema Accompanied by Changes in the Serum Proteins*

By JOHN B. VOLMANS and (by invitation) ALSTIN BELL Nashville, Tenn.

For the past three years cases of obscure edema of the legs and sometimes of the face and hands have been observed during the spring and early summer. Many gave a history of previous attacks. None of the usual causes renal disease, heart failure, et cetera were found to account for the swelling. Although the patients were well nourished and had no diseases which might have caused malnutrition, it was thought that the diet though not of a starvation type might be low in protein and the edema due to a protein deficiency. Analysis of the blood serum usually revealed a normal or increased total protein but with the serum albumin at or below the lower normal value. The globulin was increased in all cases, giving a lowered or sometimes reversed, A/G ratio. Close correlation between the presence and degree of edema and the serum protein levels was lacking, but in general the albumin or globulin increased as the edema lessened. With increases in the albumin the globulin fell. Calculated oncotic pressures were in general low during the period of edema.

*The Influence of the Thyroid on the Concentration of Protein in Cerebrospinal Fluid* By W. O. THOMPSON and (by invitation) PHILIP K. THOMPSON and MARY ELIZABETH DAILEY Boston, Mass.

In myxedema the concentration of protein in the cerebrospinal fluid is high and in toxic goiter it is low. In both the concentration returns to the normal level when the basal metabolism is restored to normal by appropriate treatment. In cases of myxedema the protein concentration is constant under the same conditions but can be varied if will by varying the level of the basal metabolism. Repeated lumbar punctures have shown that the concentration in the lumbar region in cases of myxedema returns to its initial level only very slowly (at least six days) following the withdrawal of all readily available fluid. The pressure and the quantity of circulating fluid appear to return to their initial levels before the concentration of protein

tion of iron in various forms, there is a very transient outpouring of the more mature types, followed by an increase in the more immature forms. In the hemodiagram the "shift" is to the "left" in both the blood and bone marrow in contrast to the condition in pernicious anemia where "shift" is to the "left" in the blood but to the "right" in the bone marrow. The maximum reticulocyte count is higher the lower the initial red blood cell count.

that voluntary hyperpnea caused a decrease in spinal fluid pressure in contrast to the increased pressure produced by such carbon dioxide-oxygen mixtures.

These experiments are of interest in that they offer an explanation for the dramatic effects of carbon dioxide and oxygen rebreathing in arousing patients from stupor (Loevenhart and his co-workers J Am Med Assoc 1929 Vol 88) i.e., by increasing the oxygen supply to the brain, and at the same time they suggest the possibility that some of these stupors may be due to chronic cerebral anoxemia.

*The Relative Importance of Bile Salt Toxicity and Bacterial Invasion in Bile Peritonitis* By G O BROOK and (by invitation) A P BRIGGS and ELIZABETH McGARRY, St Louis Mo

The clinical manifestations which follow introduction of whole bile or solutions of bile salts into the peritoneal cavity were described in a communication presented at the meeting of this society two years ago. Recent publications have stressed the importance of the gas-forming bacilli as etiological factors in bile peritonitis. Rewbridge has given evidence which indicates that in dogs bile peritonitis is accompanied by invasion of the peritoneal cavity by *B. welchii*.

The present report is an effort to evaluate the relative importance of infection and bile salt toxicity.

We have found that in mice and guinea pigs death is regularly caused by intraperitoneal injections of sodium taurocholate in dosage of 800 mgm per kilo body weight and frequently in dosage of 500 mgm per kilo. Death occurs in from one to forty-eight hours. In these animals we have not found bacterial invasion of the peritoneum to be an important factor. Aerobic and anaerobic cultures taken immediately after death have invariably been sterile.

In dogs similar dosages likewise cause death. Here bacterial invasion of the peritoneum occurs much more frequently. We likewise find *B. welchii* to be the organism usually present. In some instances *B. welchii* has not been found and other organisms have been present. We have been able to demonstrate toxic effects from the bile salts such as depression of blood pressure, dehydration formation of peritoneal exudate prior to invasion of the peritoneal cavity by the organism. The bacterial infection would appear to be an effect secondary to the irritation of the peritoneum by the bile salts.

*On the Nature of the Action of Iron in "Secondary" Anemia* By RAPHAEL ISAACS, CYRUS C STEPHENS and (by invitation) JOHANNES LODDFORS - GREVINICK Ann Arbor Mich

A study of three stages in the development of red blood cells (reticulocyte, granule red blood cell and mature cell) in the blood of patients with anemia of hemolytic, chlorosis and cancer suggests that following the administration

## THE RELATIONSHIP BETWEEN THE ENVIRONMENT AND THE BASAL INSENSIBLE LOSS OF WEIGHT<sup>1</sup>

By F. H. WILEY<sup>2</sup> AND L. H. NEWBURGH

(From the Department of Internal Medicine Medical School University of Michigan Ann Arbor)

(Received for publication May 22, 1931)

In 1926 Benedict and Root (1) expressed the belief that a quantitative relationship existed between the heat production in the basal state and the insensible loss of weight. General confirmation was later obtained by Levine and his colleagues (2), and by this laboratory (3).

The insensible loss is caused by the outward passage of water vapor and carbon dioxide, both of which reduce the individual's weight, but it is simultaneously augmented by the absorption of oxygen. The only component of the insensible loss of weight that carries away heat is the water vapor. The use of the insensible loss of weight as a measure of the dissipation of heat is not strictly quantitative, since this loss varies not only with the weight of the water vapor, but is also influenced by the character of the materials being metabolized. This relationship is conveniently expressed as an equation in which the weight of each substance is employed.

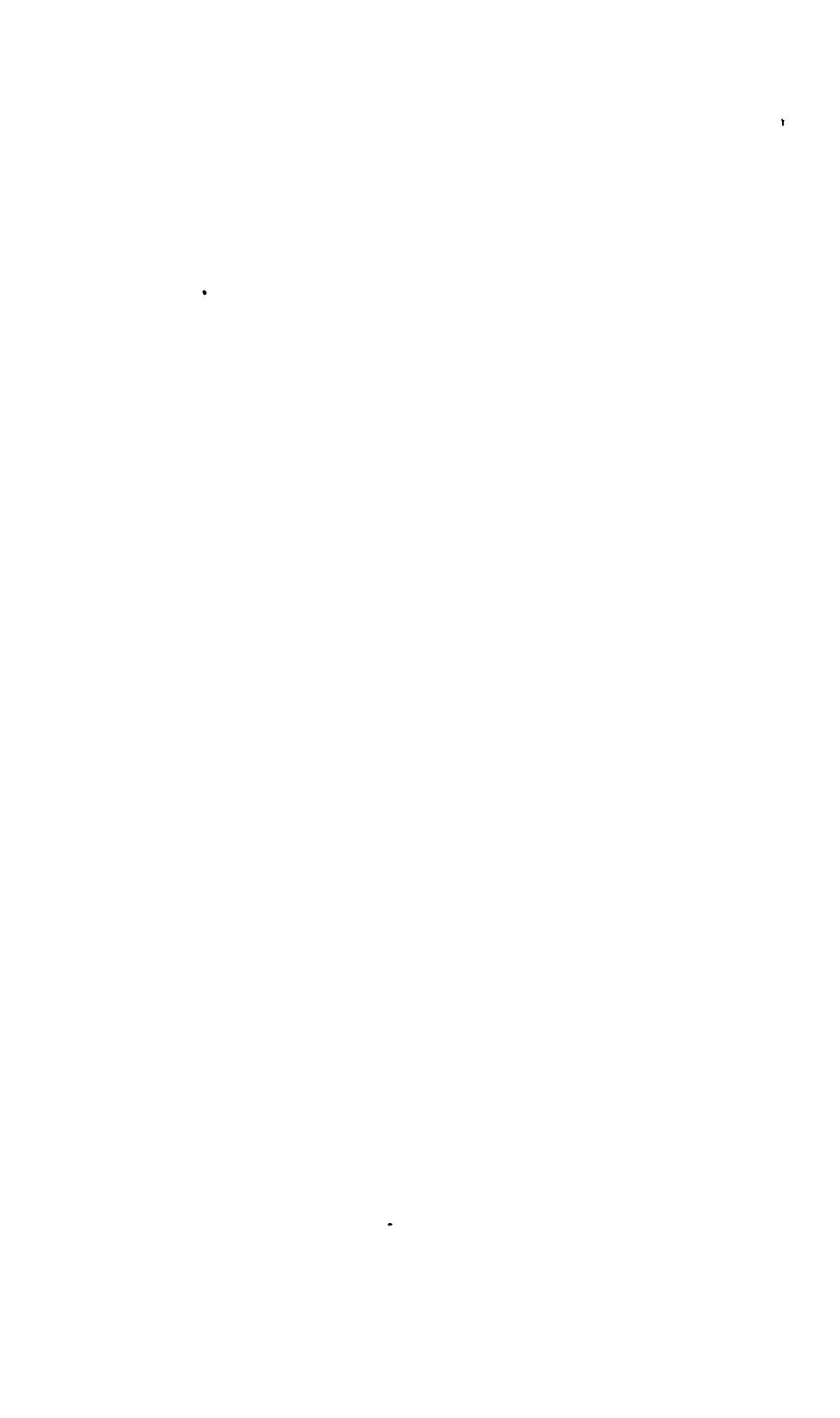


The insensible loss of weight may be obtained by means of an appropriate balance (1). If the heat production of the individual is determined by indirect calorimetry, the weights of carbon dioxide and oxygen are at hand, and these values may be used to clear the equation. Since water is now the only unknown quantity, one may, by rearranging the equation and substituting the known values, arrive at the weight of water vaporized. Thus

$$\text{H}_2\text{O} = \text{Insensible loss} - (\text{CO}_2 - \text{O}_2)$$

<sup>1</sup> The expenses of this investigation were defrayed in part by a fund for the study of nutrition created by Mr. W. K. Kellogg of the Kellogg Corn Flake Company Battle Creek, Michigan.

<sup>2</sup> National Research Council Fellow in Medicine.



calorimetry with the use of a Tissot spirometer and the Henderson-Haldane apparatus for gas analysis. The skin temperature was determined by means of a thermocouple of the usual type. The surface temperature of a number of points on the head, trunk, legs, feet, arms, and hands was obtained, and the average temperature for each of the above areas was multiplied by the fraction of the total area which it represented. The sum of the figures thus secured represents the average surface temperature of the entire body. The desired temperature and humidity of the room was obtained in the early morning and held at that level through the period required for the observations on that day. Only one set of observations was made each day, but further observations were made at the same temperature on other days.

#### *Series I Nude Subject*

*Group A Basal state Effect of atmospheric temperature.* The temperature was varied from 18° to 40° C., while the relative humidity was kept constant at 20 ( $\pm 2$ ) per cent. The results are shown graphically in Figure 1. For the sake of clarity, the rate of increase of atmospheric temperature (C) is plotted as a diagonal.

The heat production (B) followed the expected course. Between 25° C. and 37° C., the heat production was practically constant. It rose very slightly thereafter with increasing atmospheric temperature. As the temperature fell below 25° C., there was a sharp increase in heat production. At 18° C., it had increased over the basal more than 50 per cent, but was not accompanied by visible shivering.

From 18° to 30° C., the skin temperature increased at about one half the rate of increase of environmental temperature. Thereafter the rate of increase of surface temperature gradually fell off and approached a constant value of about 36° C. Between the environmental temperatures of 30° and 40° C., the skin temperature increased only 1.5° C.

A comparison between the amount of heat removed by the vaporization of water and the total heat dissipated (produced) can best be made by separating their courses into three periods. Through the range of room temperature between 18° and 25° C., the heat dissipated by the vaporization of water increased slowly at a constant rate even though the total loss of heat decreased rapidly. From 25° to 30° C., the

It has been generally understood for a long time that heat is dissipated from the body by radiation, conduction, convection, and the vaporization of water. The latter is of special interest to us since it alone can be measured by change in weight. Since in an organism in the basal state the heat dissipation and heat production are equal and since the latter may be accurately determined, we have a means of arriving at the heat dissipated. Since, in the second place, the outward passage of water vapor is accompanied by a loss of weight on the part of the organism, an accurate knowledge of the weight of the water vaporized in a unit of time immediately states the rate of loss of heat by this process, for it is true that at body temperature 0.58 Calories are removed with each gram of water vapor.

Accordingly, the determination of heat production by indirect calorimetry and of the insensible loss by means of a balance, under basal conditions, permits one to arrive at, (1) the amount of heat removed from the organism by the evaporation of water, and (2) the ratio between it and the total dissipation of heat.

Our problem consisted of recording the influence of the temperature, humidity, and movement of the air on the heat removed by the vaporization of water, and on the ratio between this value and the total heat dissipated. Since the surface temperature of an individual is not constant, but is influenced by the state of the environment, it was also necessary to record the skin temperature in order to deal with its relation to these same two values. We also wanted to know what changes, if any, are brought about by an elevation of the heat production. Finally, it is also necessary, in order to get a comprehensive understanding of the mechanism, to know the effect of clothing on the subject.

The room in which the experiments were carried out was sufficiently insulated so that the temperature and humidity could be kept at any desired level. The movement of air was obtained by an electric fan. A highly trained, presumably normal male individual was used as the subject. He was 34 years of age, 168 cm in height, and weighed 61 kilograms. The insensible loss of weight was determined by means of a balance accurate to one tenth of a gram. The subject lay on a support made of wide steel ribbons, and his head rested on a small rubber-covered pillow. The heat production was obtained by indirect

From 32° to 40° C., it rose rapidly from 35 per cent to 175 per cent. It reached 100 per cent of the dissipated heat when the skin and atmospheric temperatures were the same (i.e. 36° C.).

The events upon which the removal of heat depend may advantageously be thought of as occurring in two fields, separated from each other by an imaginary line cutting through the environmental temperature of 36° C. In the cooler field the ratio between the total dissipation of heat and that portion removed by vaporization is determined by well established physical principles and no biological factors need be employed. The increase of skin temperature, at a rate one half that of the environmental temperature, gives rise to a constantly decreasing temperature gradient between the subject and his environment and, consequently the heat lost by radiation, conduction and convection was also decreased at a constant rate. On the other hand, the rising skin temperature increased the rate of vaporization of water into an atmosphere whose relative humidity was constant. The actual increase in the vaporization of water in this field was 11 grams per 24 hours per degree change in environmental temperature.

In the warmer field, even at the left hand edge, the difference between skin and room temperatures was already so small that little heat was lost by radiation, conduction, and convection. At 36° C., these temperatures were equal, and beyond that the higher environmental temperature caused a transfer of heat from the environment to the organism. To compensate for the progressive failure of radiation, conduction, and convection to remove the heat from the organism at the proper rate the vaporization of water increased very rapidly. The physical conditions alone would not account for the observed increase in vaporization of water. (In fact, the almost constant skin temperature accounts for practically no increase in vaporization), and consequently, the observed increase is due to the active delivery of water to the skin by the sweat glands. Thus the dissipation of heat is almost entirely brought about by a physiological adaptation of the subject to his environment. The actual increase in the vaporization of water was 465 grams per 24 hours per degree change in environmental temperature, as contrasted with 11 grams in the other field. At an environmental temperature of 40° C., when the heat production was 1500 Calories, the heat lost by the vaporization of water was 2500 Calories.

removal of heat by vaporization continued to increase at the former rate even though the heat dissipation throughout this range had a fixed value. From 30° to 40° C., the removal of heat by the vaporization of water increased at a dramatic rate while the total heat dissipation remained practically constant.

Finally, the heat lost by vaporization of water expressed in per cent

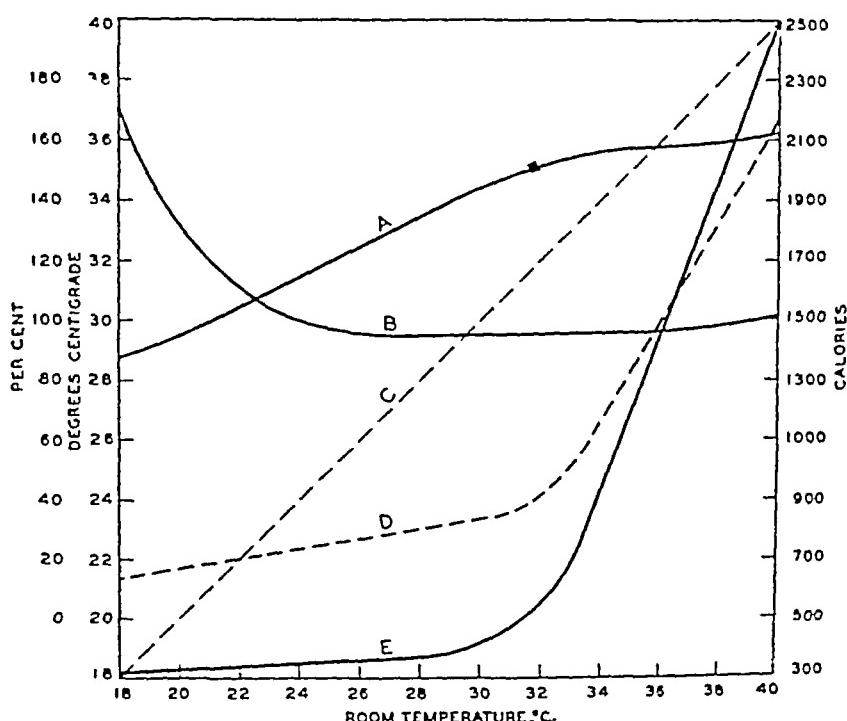


FIG. 1. NUDF SUBJECT. THE EFFECT OF ROOM TEMPERATURE ON SKIN TEMPERATURE, BASAL HEAT PRODUCTION AND THE DISSIPATION OF HEAT BY THE VAPORIZATION OF WATER AT A RELATIVE HUMIDITY OF 20 PER CENT.

A Skin temperature in degrees centigrade B Basal heat production in Calories per 24 hours C Rate of increase in room temperature D Heat lost by the vaporization of water in per cent of the total heat dissipated E Heat dissipated by the vaporization of water in Calories per 24 hours

of total heat dissipated is represented by line (D) in the figure. It will be seen that this percentile value was different at every atmospheric temperature. It increased from 15 per cent to 30 per cent of the total outgoing heat, between the atmospheric temperatures of 18° and 30° C.

the more rapid removal of heat and the consequent lowering of the skin temperature.

*Group D Effect of increased heat production* In order to observe the response of the mechanism for dissipating heat when the heat production was above the basal level, the subject was fed about 600 grams of beef each morning, about one hour before the studies began. The data were obtained during the second and third hour after ingestion of meat because this is the time when the metabolism is most likely to be maintained at the high level characteristic of the response to protein. The conditions to which the subject was exposed were otherwise the same as in "Group A" of this series. It will be seen from Figure 3 that the heat production was increased about 40 per cent over the basal, and showed the same tendency to increase still further in response to cold as was observed in the basal state. In spite of the increase of the heat production the percentile ratio between this value

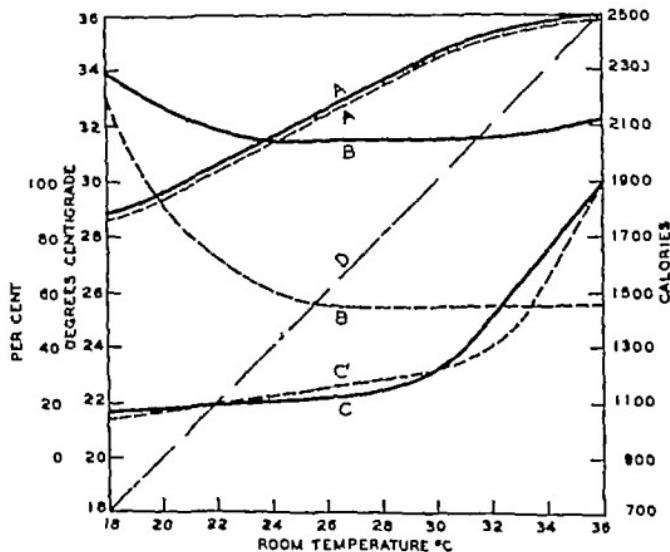


FIG. 3 NUDE SUBJECT THE EFFECT OF AN INCREASED HEAT PRODUCTION ON THE SKIN TEMPERATURE AND THE DISSIPATION OF HEAT BY THE VAPORIZATION OF WATER AT A RELATIVE HUMIDITY OF 20 PER CENT

A Skin temperature in degrees centigrade B The heat production in Calories per 24 hours C Heat lost by the vaporization of water in per cent of the total heat dissipated D Rate of increase in room temperature. A', B' and C' represent the values found in the basal state (See Fig. 1)

The extra 1000 Calories represented the absorption of heat by the individual from the hot air of the room.

*Group B Basal state Effect of humidity* The effects of relative humidity ranging from 20 per cent to 80 per cent were recorded. The room temperature was maintained at 28° C. These effects are represented in Figure 2. The skin temperature throughout this group

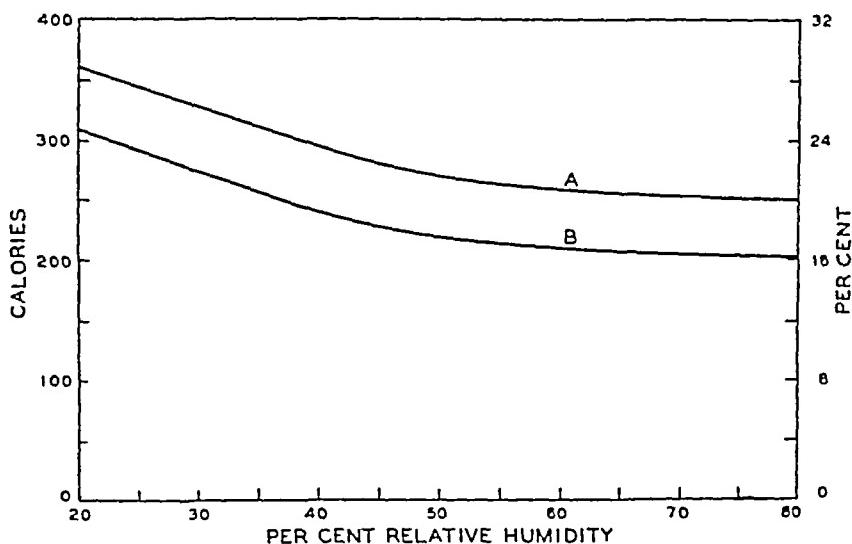


FIG. 2 NUDE SUBJECT THE EFFECT OF RELATIVE HUMIDITY ON THE DISSIPATION OF HEAT BY THE VAPORIZATION OF WATER AT AN ENVIRONMENTAL TEMPERATURE OF 28° C

A Heat lost by the vaporization of water in per cent of the total heat dissipated. B Heat dissipated by the vaporization of water in Calories per 24 hours.

remained constant at 33.5° C ( $\pm 0.2^\circ$ ). The heat production was uninfluenced by the humidity. It is quite clear that an increasing relative humidity of the atmosphere caused a decreasing vaporization of water from the surface of the skin, other things being equal. Since the skin temperature and heat production did not change, the organism was in no way responsible for this phenomenon.

*Group C Basal state Effect of wind* When a powerful air current was directed against the nude subject, the amount of water vaporized in a unit of time was definitely less than when the air was still and other conditions were unchanged. This decrease can be attributed to

*Series II Clothed Subject Basal State*

*Group A Heavy "Canton" flannel pajamas with feet, and without mattress or blankets* The effects of environmental temperature on the subject thus clad were recorded in the same way as in Series I-A. The results are represented in Figure 4. The effect of clothing can be ob-

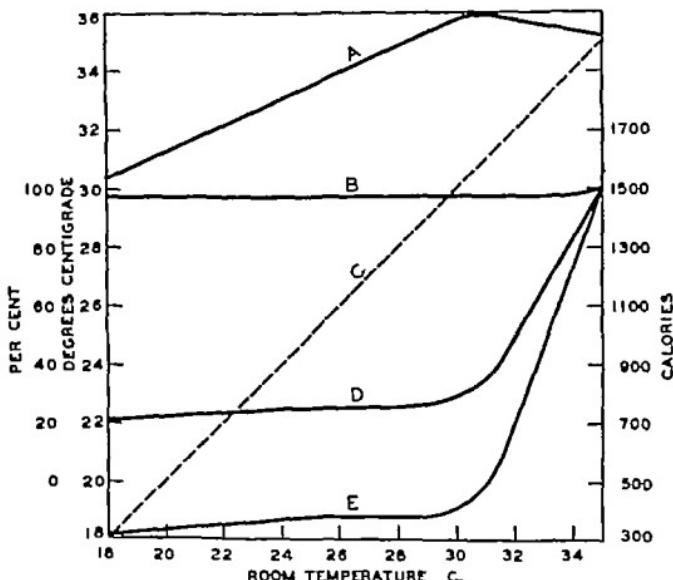


FIG 4 CLOTHED SUBJECT THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE SKIN TEMPERATURE, BASAL HEAT PRODUCTION, AND THE DISSIPATION OF HEAT BY THE VAPORIZATION OF WATER

A Skin temperature in degrees centigrade B Basal heat production  
 C Rate of increase in room temperature D Heat lost by the vaporization of water in per cent of the total heat dissipated E Heat dissipated by the vaporization of water in Calories per 24 hours

tained by comparing Figures 1 and 4, since all other conditions were the same. When the air was cold the protection afforded by clothes permitted a skin temperature high enough so that the organism did not produce extra heat. The significant difference between the nude and clothed states is the tendency for the amount of heat removed by the vaporization of water to approach constancy between the atmospheric temperatures of 18° and 30° C when the subject is clothed. In fact,

and the amount of heat dissipated by the vaporization of water followed the same general plan as it did in the basal state, but differed from the latter in two definite respects (1) The active participation of the sweating mechanism began at a temperature about  $4^{\circ}$  lower, (2) during the range of temperature below the sweating level, the amount of heat dissipated by the vaporization of water showed a greater tendency to approach constancy than in the basal state After meat, as in the basal state, all of the heat was carried away by vaporization when the atmospheric temperature was  $36^{\circ}$  C, since the skin temperature in both cases had also attained this level at that temperature

#### *Discussion*

The study of the effects of the atmosphere on the nude subject in the basal state has clearly shown that increasing temperature of the air is accompanied by an increasing loss of heat by vaporization of water, and that this increase is first slow and then abruptly becomes rapid The second fact is the almost uniform decrease in the amount of heat removed by the vaporization of water as the relative humidity increases

When the heat production is markedly increased above the basal level, the mechanism for the removal of heat conducts itself, broadly, in the same way as when the heat production is at the basal level However, the activity of the sweat glands is brought into play at a lower temperature Consequently, the purely physical mechanism fails sooner when extra heat is produced

If it were intended to measure, in the nude basal subject, the total dissipation of heat, or its counterpart, the heat production, by means of the weight of the vaporized water, it would be essential to construct a prediction table of the per cent of the total heat lost by vaporization of water at every atmospheric temperature and for every relative humidity in still air Furthermore, it would be desirable to restrict the temperature to the five degree range between  $26^{\circ}$  and  $31^{\circ}$  C, since below  $26^{\circ}$  C there is a rising heat production, and above  $31^{\circ}$  C the increase in heat removed by vaporized water is so rapid that reliable values could scarcely be expected No one has yet constructed such a table

It was found that, at an environmental temperature of 25° C., a variation of relative humidity from 20 per cent to 60 per cent did not change the amount of heat removed by the vaporization of water. The observations also showed that the heat removed by water vapor tended to be constant from day to day for this individual when the environmental temperature and the bedding were constant.

### *Series III Various Subjects*

It remained to be determined whether a series of individuals under the same conditions, would lose a constant proportion of the dissipated heat by vaporization of water. The bedding consisted of a mattress covered with a rubber sheet and a blanket, and an additional blanket was over the subjects. They wore light pajamas. The room temperature was kept at 25° C. Table I sets forth the data thus obtained.

TABLE I

*Heat dissipation by the vaporisation of water for a group of subjects under constant environmental conditions*

Subject	Height	Weight	Sex	Heat lost by vaporisation of $H_2O$ in per cent of total
L B Diabetic—Age 9	125 cm	Weight 25.65 kgm	Sex—F	24.6
G B Nephritic—Age 10	147 cm	Weight 26.9 kgm	Sex—M	24.0
C J P Nephritic—Age 16	167 cm	Weight 58 kgm	Sex—M	28.8
M K. Normal—Age 23	165 cm	Weight 70.94 kgm	Sex—F	27.3
H M K. Obese—Age 29	180 cm	Weight 99 kgm	Sex—M	32.0
F. H. W. Normal—Age 28	180 cm	Weight 61 kgm	Sex—M	25.2
F D J. Diabetic—Age 30	174 cm	Weight 56 kgm	Sex—M	28.2
L J. H. N. Normal—Age 47	168 cm	Weight 54 kgm	Sex—M	19.3
Average				21.9
				31.9
				21.7
				30.5
				34.0
				27.2

It will be seen that the heat dissipated by vaporization, under these constant conditions varied from 19 per cent to 34 per cent of the total heat dissipated. The average value was 27.2 per cent, and the variation from it ranged between -30 per cent and +25 per cent. When

the heat removed by vaporization is constant for the 7 degree range between 23° and 30° C., from the subject thus clad

*Group B Varied kinds of clothing* Since the setting up of a barrier between the subject and the environment produced the above described effect, it became desirable to know whether the further addition of mattress and bedding would widen the temperature range through which a constant amount of the heat would be removed by the vaporization of water

Throughout this series the subject lay on a mattress which was at times enclosed in a rubber sheet. The subject himself sometimes wore light pajamas, and at other times he wore heavy ones. We tried the effect of covering the mattress with a cotton sheet or with a blanket. The subject was uncovered, or was covered with a sheet, or one or two blankets. With none of these combinations did we obtain satisfactory results.

It seemed possible that the irregular results were caused by the loss or gain of weight of the bedding during the period of observation. In order to overcome this source of error, the following procedure was employed. The subject lay quietly for a half hour, or longer, in the bed. He was then cautiously removed and sat upon an adjoining chair, appropriately covered, while the observer determined the weight of the bed. The subject was then returned to the latter and the insensible loss of weight of the system, of which he was a part, was obtained in two or three successive intervals, which together amounted to about one half hour. After again removing him from the balance, the weight of the bed was obtained a second time. The bed was found to lose or gain from one to ten grams per hour in an irregular manner. The insensible loss of the subject was then obtained by correcting the total insensible loss for this value. This method for correcting for change in the weight of the bed was employed in all subsequent observations.

*Group C Effect of humidity* Since both temperature and humidity affect the rate of insensible loss of the nude subject, it was necessary to study the effect of one of these under the new conditions, while the other remained constant. We chose to vary humidity, while a constant environmental temperature was maintained. The bedding consisted of a mattress covered with a rubber sheet and a blanket, and an additional blanket was over the subject. He wore light pajamas throughout.

# A METHOD FOR THE DETERMINATION OF HEAT PRODUCTION OVER LONG PERIODS OF TIME<sup>1</sup>

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Heretofore, it has not been possible to measure the heat production of individuals leading their usual lives, due to the unavoidable restrictions necessary to carry out either direct or indirect calorimetry. It is quite obvious that the movements of a subject in a calorimeter are sharply restricted by the capacity of the apparatus and, hence, prevent normal activity. On the other hand, the value of determinations based on indirect calorimetry depends on sampling in such a way that the expired air thus collected is really representative of the period, and there is no way of knowing whether this is the case. Further, the sampling necessarily requires the individual to be at rest for frequent ten minute periods and so disturbs his usual routine. The method which we are proposing is not hampered by either of these two restrictions.

In an earlier paper (1) it was pointed out that the insensible loss of weight is roughly proportional to the heat production of the period, provided two sources of error are excluded namely, first, there must be a proper relationship between the rate of heat production and the environmental conditions and, secondly, the subject must be transforming a minimal amount of energy to mechanical work. In the previous paper it was also shown that the insensible loss of weight is the resultant of the weight of water lost by evaporation, the weight of exhaled carbon dioxide and the weight of absorbed oxygen. The relationship can be conveniently expressed as an equation

$$\text{Insensible loss of weight} = \text{H}_2\text{O} + \text{CO}_2 - \text{O}_2$$

<sup>1</sup> The expenses of this investigation were defrayed in part by a fund for the study of nutrition created by Mr W K Kellogg of the Kellogg Corn Flake Company Battle Creek Michigan

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The experiments now to be described were designed to determine whether an individual leading his normal existence would lose a fixed per cent of his total heat production by the vaporization of water. The weight of the vaporized water was multiplied by 0.58 to get its equivalent in heat units and this latter value was then compared percentilely with the total heat production.

In order to compare the heat dissipated by vaporization of water with the total heat produced it was, of course, essential to know how much heat was produced. The energy transformations of an individual who has been on a constant diet for a long period of time will necessarily come into balance with the diet and thereafter the heat production will equal the available energy of the diet. If an individual whose food has been unrestricted is now placed on a diet whose available energy is not far from his metabolic needs, his transformations of energy will shortly be in balance with the energy of the diet. When this point has been reached the observer will have at hand a knowledge of the average heat production of the individual. If he now obtains the weight of the water vaporized it will be a simple matter to calculate what proportion of the transformed energy was lost in this manner.

Day to day changes in body weight cannot be used to decide whether the subject's transformation of energy is greater or less than the available energy of the diet due to the irregular hydration and dehydration of the tissues. On the other hand the *trend* of the weight is significant. A persistent loss or gain in the body weight over a long period of time can be taken to indicate a loss or deposition of body tissue. The longer the individual has adhered to a fixed plan the greater the probability that his energy transformations will be the same as the inflow of energy.

After the subject is in balance with a diet, it is necessary to know as closely as possible what the actual energy value of the diet is. Repeated analyses in this laboratory over a number of years have shown that the available energy of the ordinary mixed diet, prepared in our special diet kitchen, is two to three per cent less than the prescribed value. The major part of this deficiency occurs in the fat portion (probably due to loss in serving).

After we had taken pains to arrive at as close a value as possible for

The numerical relationship of the three factors making up the insensible loss of weight was discussed and it was shown that, given constant weight of vaporized water, the insensible loss of weight varies with the respiratory quotient.<sup>3</sup> Since the vaporization of water is the only factor in insensible loss of weight that removes heat from the body, it, and not the total insensible loss, should be used when dealing with the dissipation of heat. Furthermore, it is true that the dissipation of heat keeps pace with the heat production when there is no change in body temperature and, that the heat production is equal to the total energy transformations when no energy is being converted to mechanical work. Accordingly, if a fixed per cent of the total heat produced is carried away by evaporation of water, then the weight of this water vapor may be used as a measure of the total heat production, or its equivalent, in the absence of mechanical work,<sup>4</sup> the total transformation of energy.

<sup>3</sup> Respiratory quotient	Insensible water grams	Insensible loss of weight grams
1.00	978	1193
0.82	978	1054
0.707	978	940

<sup>4</sup> "Mechanical work." This term as used in calorimetry means the transfer of energy from one system to another. The animal body is made up of two systems, one, the mass of the body and, two, a machine for transforming energy. Therefore, the organism may either do work upon itself, i.e. its mass, or upon some external object. Horizontal motion on the part of the animal organism is accompanied by a negligible amount of mechanical work since the resistance to this type of motion is very small. On the other hand vertical motion requires mechanical work in proportion to the product of weight and distance. In this case all of the energy transformed does not appear as heat, since some of it is stored as energy of position. But in the course of the day's routine, he who goes up comes down again, and this energy of position is lost in the form of heat. Only when the individual is further from the center of gravity at the end of the period than he was at the beginning, has he stored any extra energy of position in himself.

When an individual lifts a weight to a higher position, and leaves it there, or when he supplies the energy to drive a machine, he does mechanical work. The heat equivalent of mechanical work is very small. One caloric will lift 427 kgm one meter. It is accordingly evident that, excepting those who earn their livelihood as laborers, human beings do not convert a significant amount of the energy liberated in the body to mechanical or



period of sixty-nine days she received 2000 Calories per day and her weight again fell to 53.6 kgm. Accordingly, this subject lost weight on diets containing 1800 and 2000 Calories per day and gained weight when the energy intake was 2200 Calories per day. A consideration of these values suggests that she was transforming energy at a rate of more than 2000 Calories and less than 2100 Calories per day.

She was now placed on the same type of diet whose energy value was 2000 Calories, and the first period, during which the twenty-four hourly insensible loss of weight was determined, began on the morning of December 22, 1930. She remained on this diet for eighty-five days and lost 1.1 kgm., or 13 grams per day. Table I sets forth the data.

TABLE I  
*The relationship between total heat production and the insensible loss of water,  
Subject II*

Days on diet	Daily insensible H <sub>2</sub> O	Days on diet	Daily insensible H <sub>2</sub> O	Days on diet	Daily insensible H <sub>2</sub> O
1	grams 1044	22	grams 1125	79	grams 1059
2	666	23	1167	80	1124
3	876	24	1066	81	1176
5	916	25	956	82	1033
6	930	26	966	83	948†
7	840	27	966	84	1011
8	1222*	28	825	85	913
		29	956		
Averages	970		1003		1037

Diet (2000 Calories) + body fat (75 Calories) = 2075 Calories transformed

Average insensible water = 1003 grams

$1003 \times 0.58 = 582$  Calories dissipated by vaporization

$582 \over 2075 = 28.0$  per cent of total heat removed

Weight on first day of diet 52.6 kgm

Weight on last day of diet 52.4 kgm

\* Extra work (Not included in average)

† Sunday

*Subject III T M* Male, diabetic, age 56 years, weight 70 kgm, height 180 cm, basal metabolism 1640 Calories. This subject had been on a weighed diabetic diet containing about 2525 Calories per day for six months, without any significant change in body weight. On December 31,

1930 when his weight was 70.58 kgm, his diet was changed so it contained about 2425 Calories. In twenty days on this diet his loss of weight was 2.8 kgm half of which was lost on the first day. He was then returned to his original diet and in fifty two days he gained 1.5 kgm. From these figures it is obvious that his transformation of energy is about 2500 Calories per day. His average insensible water (Table II), omitting days on which

TABLE II

*The relationship between total heat production and the insensible loss of water,  
Subject III*

Days on diet	Daily insensible $H_2O$								
1	grams 1018	8	grams 882*	15	grams 833*	63	grams 1178	70	grams 967
2	957	9	1009	16	904*	64	913	71	1077
3	990	10	1055	17	929*	65	1056	72	923
4	1143	11	1065	18	929*	66	1033		
5	878*	12	1014	19	1045	67	1151		
6	1083	13	1011	20	954	68	933		
7	1059	14	846*	21	860*	69	957		
Averages	1042		1031				1031		

Diet (2500 Calories) = Calories transformed

Average insensible water = 1026 grams.

$1026 \times 0.58 = 595$  Calories dissipated by vaporization

$\frac{595}{2500} = 23.8$  per cent of total heat removed

Weight on first day of diet 70.6 kgm

Weight on last day of diet 70.2 kgm

\* Too cold (Not included in average.)

he was known to have been too cold, was 1026 grams per day. This value multiplied by 0.58 indicates a loss of 595 Calories per day by the vaporization of water, which is 23.8 per cent of the total heat production.

*Subject IV RLG* Male graduate student age 24 years, weight 66 kgm, height 174 cm basal metabolism 1700 Calories. His average insensible loss of weight over a period of one week suggested that he was transforming about 3600 Calories per twenty four hours. Accordingly a diet containing 3700 Calories was prescribed. Recalculation according to the principles discussed above indicated that he was actually receiving close to 3650 Calories per twenty four hours. He remained on this diet for sixty eight days. During these sixty-eight days he added 1000 grams to his body

weight If this were solely adipose tissue he would have deposited 125 Calories daily in that form However, it is probably true that some glycogen and protein were also deposited These substances per gram of wet weight contain much less energy than adipose tissue It would be safe to assume that the subject transformed 100 fewer Calories than the energy available in the diet (See Table III )

TABLE III

*The relationship between total heat production and the insensible loss of water,  
Subject IV*

Days on diet	24 hourly insensible H <sub>2</sub> O	Days on diet	24 hourly insensible H <sub>2</sub> O	Days on diet	24 hourly insensible H <sub>2</sub> O
22	grams 1813	36	grams 1442	62	grams 1581
23	1605	37	1467	63	1927†
24	1655	38	1542	64	1657
25	1639	39	1504	65	1253‡
26	1451	40	1362*	66	1730
27	1456	41	1534	67	1602
28	1679	42	1663	68`	1437
Averages	1614		1502		1551

Diet (3650 Calories) — deposition (100 Calories) = 3550 Calories transformed

Average insensible water = 1544 grams §

$1544 \times 0.58 = 895$  Calories dissipated by vaporization

$\frac{895}{3550} = 25.2$  per cent of total heat removed

Weight on first day of diet 66.5 kgm

Weight on last day of diet 67.5 kgm

\* Sunday

† Sweat (Not included in average)

‡ Afternoon nap

§ Insensible water, 1614 for 14 days, 1526 for 54 days

*Subject V A W* Graduate student, male, age 23 years, weight 63.5 kgm, height 165 cm, basal metabolism 1600 Calories His average insensible loss of weight over a period of ten days suggested that he was transforming about 3600 Calories for twenty-four hours Accordingly he received a diet containing 3650 Calories in twenty-four hours He remained on this diet for sixty-five days During this period he gained 800 grams, which, according to the principles discussed under Subject IV, indicates a deposition of about 80 Calories daily The data are to be found in Table IV

TABLE IV

*The relationship between total heat production and the insensible loss of water  
Subject V*

Days on diet	24 hourly insensible H <sub>2</sub> O	Days on diet	24 hourly insensible H <sub>2</sub> O	Days on diet	24 hourly insensible H <sub>2</sub> O	Days on diet	24 hourly insensible H <sub>2</sub> O
20	grams 1827	34	grams 1539	38	grams 1314*	59	grams 1529
21	1515	35	1723	39	1385	60	1714†
22	1505	36	1715	40	1439	61	1766
23	1673	37	1640	41	1648‡	62	1279
24	1581			42	1402	63	1510
25	1439			43	1352	64	1698
26	1776			44	1492	65	1688
Averages	1617		1654		1433		1578

Diet (3650 Calories) - deposition (80 Calories) = 3570 Calories transformed

Average insensible water = 1538 grams

$1538 \times 0.58 = 882$  Calories dissipated by vaporization.

$\frac{882}{3570} = 24.7$  per cent of total heat removed

Weight on first day of diet 63.7 kgm

Weight on last day of diet 64.5 kgm

\* Very quiet day

† 3 hours sleep

‡ Perspiration (Not included in the average)

*Subject VI MP* Medical student male, age 25 years, weight 67.3 kgm height 175 cm basal metabolism 1695 Calories This subject was placed on a diet of 3425 Calories and left on it throughout the study During the first thirty five days there was no significant change in weight Two weekly periods of insensible water were obtained during this interval Table V sets forth this data During a subsequent period of thirty two days he gained 1400 grams Both the subject and we were aware that he was less active throughout this interval The average insensible water for a week at the end of the second period was 1296 grams indicating a transformation of 3150 Calories and permitting a deposition of 275 Calories daily This decrease in insensible water is significant, since the gain in weight without change of diet indicated a diminished transformation of energy

*Subject VII MW* Female chemist, age 24 years weight 73 kgm, height 168 cm basal metabolism 1615 Calories This subject was put on a diet containing about 2800 Calories and the daily insensible loss of weight

TABLE V

*The relationship between total heat production and the insensible loss of water,  
Subject VI*

Days on diet	Daily insensible H <sub>2</sub> O	Days on diet	Daily Insensible H <sub>2</sub> O
1	grams 1357	28	grams 1383
2	1292	29	1423
3	1415	30	1700*
4	1349	31	1501
5	1411	33	1447
6	1645	34	1413
7	1518	35	1512
Averages	1425		1426

Diet (3425 Calories) = Calories transformed

Average insensible water = 1425 grams

$1425 \times 0.58 = 826.5$  Calories dissipated by vaporization

$\frac{826}{3425} = 24.1$  per cent of total heat removed

Weight on first day of diet 67.3 kgm

Weight on last day of diet 67.6 kgm

\* Additional activity (Not included in average)

was obtained for one week. It indicated, on the assumption that 24 per cent of the heat production was dissipated by the vaporization of water, that her daily transformation of energy was about 3230 Calories. During this period she lost weight very sharply.

She was then placed on a diet containing about 3150 Calories. Her weight did not change significantly during the next two weeks, at which time she acquired an acute infection, causing the experiment to be interrupted for sixteen days. During this interval she gained weight. She now began to live on the experimental diet again, and her weight at the beginning was 73.68 kgm. She lost weight slowly on this diet, as was to be expected, since the insensible loss of water for a second period of one week indicated a transformation of 3215 Calories per day. After two weeks on this diet, her calorific intake was increased to 3225 Calories. After four weeks on this last diet, her weight was back to the original level. These considerations indicate that an average daily intake of 3210 Calories was necessary for maintenance of weight.

*Subject VIII* Male chemist, age 28 years, height 183 cm, weight 57 kgm, basal metabolism 1470 Calories. This subject was placed on a diet containing 2922 Calories per day. Since this period was to be used as the

TABLE VI

*The relationship between total heat production and the insensible loss of water,  
Subject VII*

Days on diet	Daily insensible H <sub>2</sub> O	Days on diet	Daily insensible H <sub>2</sub> O
1	grams 1433	38	grams 1435
2	1769*	39	1253
3	1190	40	1500
4	1348	41	1220
5	1439	42	1363
6	1135	43	1585
7	1434	44	1220
Averages	1330		1368

Diet (3210 Calories) = Calories transformed

Average insensible water = 1349 grams.

$1349 \times 0.58 = 781$  Calories dissipated by vaporization

$\frac{781}{3210} = 24.4$  per cent of total heat removed

Weight on first day of diet 73.7 kgm

Weight on last day of diet 73.7 kgm

\* Extra work (Not included in average)

basis for comparison in another study, we took great pains to get the actual available energy of the diet by use of the bomb calorimeter. He was found to be in nitrogen balance and since his daily variation in weight was so slight and the total change over a period of eighteen days was only 14 grams it is safe to conclude that he was also in caloric balance. Calculation of water exchange showed that he was in balance in this respect also, and that a loss or gain of tissue had not been concealed by a shift in water. A graph of his weight record, corrected for daily shifts in water exchange is a straight line. His total insensible water for eighteen days was 21,964 grams, and this multiplied by 0.58 gave 12,739 Calories or 708 Calories per day lost by the vaporization of water. This is 24.2 per cent of the total energy transformation.

#### DISCUSSION

Our earlier study (3) in this field clearly showed that (1) the amount of heat lost by the vaporization of water in the nude, resting subject was strikingly affected by the temperature and the humidity of the environment (2) that the effect of humidity was negligible when the subject was clothed, and that the effect of temperature was less marked,

and (3) that, in the short basal periods with clothed individuals in the presence of a fixed environmental temperature, the amount of heat lost by the vaporization of water varied from about 20 to 34 per cent of the total heat dissipated. Even though this method is not adapted to measuring heat production accurately for brief intervals of time, there is, *a priori*, no reason why it should not be successful as a means of obtaining the total exchange of energy over protracted periods.

Our investigations have emphasized the necessity of guarding against two sources of error. We have found that when the individuals become unusually warm, the ratio between the heat lost by vaporization of water and the total heat production increases markedly above the average and returns to the usual value when the condition is corrected. This condition may be due either to an increase in the environmental temperature or to an increase in the heat production, and can often be corrected by an adjustment in the amount of clothing. In any case the individual is generally aware of being uncomfortably warm. For example, in Table III, on the sixty third day, the subject, following his usual routine, unwittingly wore heavy winter clothing while walking out of doors on a day strikingly warm for the season. He was conscious of perspiring freely and his insensible loss of water rose from an average of 1550 grams per twenty-four hours to 1927 grams. Thus a subject in an experiment of this type should be warned to prevent himself from becoming uncomfortably warm. On the other hand, an unusually large value for the insensible water of a twenty-four hour period does not necessarily mean that the per cent of heat dissipated by it is out of proportion to the average per cent. It may simply be due to an increased heat production. Thus in Table VI, on the second day, the subject moved to a new abode and the accompanying increased activity was reflected in the insensible water for the period.

The opposite effect has been observed when the individual becomes uncomfortably cool. A striking example of this effect is seen in Table II from the fourteenth to the eighteenth day inclusive. During a period of unusually cold weather, the subject was either too obstinate or too indolent to put extra bedding over himself at night. He preferred the alternative of having a series of disturbed nights due to being uncomfortably cool. Obviously this error is more easily avoided than the first.

All of these subjects were exposed to the wide variations in environmental temperature and humidity between the heated buildings and the cold out of doors during the winter, but they gave no more than the habitual attention to keeping their bodies comfortable. Nevertheless, the daily insensible loss of water for each individual remained within a narrow range.

The individual following his usual routine tends to expend the same amount of energy from week to week rather from day to day. It is accordingly more significant to compare the weekly averages of insensible water than to compare the daily averages. Thus for three individuals whose routine was very constant, the weekly averages were (Table II) 1042, 1031, 1031 (Table V) 1425, 1426, and (Table VI) 1330, 1368. In the case of the two graduate chemical students there was a change in activity in the middle of the period of observation, and the subjects predicted a smaller insensible loss of water for the latter part of the period. Thus in Table III there is a fall from 1614 grams to 1502 grams and 1551 grams on subsequent weeks, and in Table IV the weekly losses for the first part of the period are 1617 grams and 1654 grams, and in the latter portion of the period they are only 1433 grams and 1578 grams. This is a very satisfactory agreement between the activity of the individual and the dissipation of heat by the vaporization of water.

The average per cent of the heat production removed by the vaporization of water for each individual will be found in Table VII. A

TABLE VII

*Percentile relationship between total heat produced and heat removed by vaporization of water*

Subject	Total transformation of energy	Heat lost by evaporation of H <sub>2</sub> O	Surface area	Remarks
L R.	calories 2075	per cent 28.0	square meters 1.49	Diabetic—Nurse
T M	2500	23.8	1.89	Diabetic—Male
F D J	2995	24.2	1.67	Diabetic—Intern
F H W	2920	24.2	1.73	Chemist—Male
M W	3150	24.4	1.79	Chemist—Female
M P	3425	24.1	1.81	Medical Student—Male
A W	3650	24.7	1.69	Graduate Student—Male
R. L G	3650	25.2	1.80	Graduate Student—Male

survey of the table shows that the results from all but one of the eight subjects fall within rather narrow limits. In the case of L R., we soon recognized that her occupation was such that bursts of intense extra effort could not be avoided and these periods were undoubtedly accompanied by sweating. Obviously, then, the method is not applicable to an individual under these conditions. Accordingly, we shall omit from further considerations the figures obtained in this case. The average value for the remaining seven is 24.4 per cent. Thus each of seven of the subjects, the data from whom appeared to be satisfactory, dissipated very close to 24 per cent of the total heat by the vaporization of water. On the basis of these results we feel that the measurement of insensible loss of water, observing the precautions set forth above, furnishes a satisfactory determination of the total exchange of energy in the case of the unrestricted individual.

At present there is no reason for believing that this method is not applicable to patients lying comfortably in bed, but no data are at hand. Whether the method has any value in febrile or painful disease, is not known.

#### APPLICATION

It has been pointed out above that the weight of the vaporized water is proportional to the energy exchange, and that this weight may be calculated from the insensible loss of weight when the weight of carbon dioxide and oxygen is known. Since we strove to feed this group of subjects a diet whose calorific value equaled the heat production, it was a simple matter to calculate the carbon dioxide and the oxygen values directly from the composition of the diets by means of the constants listed on page 706.

However, it is also quite possible to calculate the carbon dioxide and oxygen exchange of an individual on any diet, or even in starvation, provided the magnitude of the carbohydrate ingestion is known and the nitrogen excretion is determined. The procedure for arriving at the difference between the weights of carbon dioxide and oxygen, and the magnitude of the heat production, for a period under any of these conditions, may be conveniently illustrated by means of hypothetical examples.

*I. Starvation.* Within the first few days of starvation, the liver glycogen has been destroyed. Thereafter, the metabolic mixture con-

sists of protein and fat, the protein of which can be determined from the nitrogen excretion. The remainder of the energy comes from the oxidation of fat.

An individual is assumed to lose 912 grams insensibly in twenty four hours, and to excrete 8.1 grams of nitrogen during this time. The protein destruction is therefore 50.6 grams and 202 Calories were liberated from it. Since the subject is starving, the respiratory quotient will be close to 0.72. At that quotient the weight of the carbon dioxide equals that of the oxygen, hence, under these conditions, the insensible loss of weight is made up entirely of the weight of the insensible water. Accordingly the insensible loss of weight during starvation may be used directly to get the first approximation of the energy exchange for the period. Thus

$$(1) \frac{912 \times 0.58}{0.24} = 2204 \text{ Calories energy exchange (First approximation)}$$

$$(2) 2204 - 202 = 2002 \text{ Calories from fat (First approximation)}$$

$$(3) \frac{2002}{9.54} = 210 \text{ grams fat (First approximation)}$$

$$(4) \text{CO}_2 - \text{O}_2$$

$$50.6 \text{ (protein)} \times 0.08 = 4.0 \text{ grams}$$

$$210 \text{ (fat)} \times (-0.08) = -17.0 \text{ grams}$$

$$\underline{\hspace{10em}} -13.0 \text{ (Second approximation)}$$

$$(5) 912 \text{ (Insensible loss)} - (-13) = 925 \text{ Insensible water}$$

$$\text{(Second approximation)}$$

$$(6) \frac{925 \times 0.58}{0.24} = 2236 \text{ Calories energy exchange (Second approximation)}$$

The second approximation yields a sufficiently accurate answer, since the third approximation will be within 0.5 per cent of the second one.

*II Submaintenance.* When a subject is being undernourished, a depletion of liver glycogen also takes place and within a few days the carbohydrate of the metabolic mixture is equal to that of the diet. The subject must accordingly come into carbohydrate balance before precise values for energy exchange can be obtained by this method.

An individual is assumed to be receiving the following diet protein 70 grams, fat 100 grams, carbohydrate 100 grams. The insensible loss of weight is assumed to be 1027 grams per twenty-four hours, with a nitrogen excretion of 11.8 grams. Hence, 74 grams protein were oxidized, yielding 296 Calories. Since the subject had attained carbohydrate balance he was oxidizing 100 grams carbohydrate per twenty-four hours, from which he obtained 400 Calories.

From inspection of the constants previously cited, it is evident that the greatest portion of the difference between the weights of the carbon dioxide and oxygen is due to the combustion of carbohydrate, and, since the effect of fat and protein upon this difference tend to cancel each other, a first approximation of this difference may be obtained by considering only the carbohydrate.

- (1)  $\text{CO}_2 - \text{O}_2$  (First approximation)  
 $100 \text{ grams carbohydrate} \times 0.41 = 41 \text{ grams}$
- (2)  $1027 \text{ (Insensible loss)} - 41 = 986 \text{ grams insensible water}$   
 (First approximation)
- (3)  $\frac{986 \times 0.58}{0.24} = 2383 \text{ Calories energy exchange}$  (First approximation)
- (4)  $2383 - 296 \text{ (Calories from protein)} - 400 \text{ (Calories from carbohydrate)} = 1687 \text{ Calories from fat}$  (First approximation)
- (5)  $\frac{1687}{0.54} = 177 \text{ grams fat}$  (First approximation)

(Second approximation)

$$\begin{array}{rcl} " & \times (-0.08) = & -14 \\ & " \text{ (carbohydrate)} \times 0.41 = & 41 \\ & \times 0.08 = & 6 \\ & & \hline \end{array}$$

33

Insensible water (Second approximation)

Change (Second approximation)

tly accurate

*III Maintenance* In this case, over any protracted period, the carbohydrate of the metabolic mixture will be the same as that of the diet. The small inequalities on single days will balance each other over any few days. Accordingly, the difference between the weights of the carbon dioxide and oxygen may be calculated from the diet.

*IV Supermaintenance* The extra energy of such a diet may be deposited as any of the food stuffs. Since the amount of protein oxidized is determined by nitrogen excretion deposition of extra protein does not concern us in the calculation of the metabolic mixture.

After a few days it may be safely assumed that no more glycogen will be deposited. Thereafter the extra energy contained in the carbohydrate and fat of the diet will be deposited as fat. Further, for purposes of calculation, it may be properly assumed that the carbohydrate of the diet is completely oxidized. However, it makes no difference in the calculation if a portion of the dietary carbohydrate is deposited as fat and the equivalent energy obtained from the oxidation of fat, as will be shown below.

Let us assume that a subject receives 100 grams of glucose and completely oxidizes it. The  $\text{CO}_2 - \text{O}_2$  for it would be  $146.7 - 106.7 = 40$  grams. Let us next assume that when the same amount of heat is liberated, fifty per cent of it comes from the oxidation of the glucose itself that the remainder of the glucose is first converted to fat and that the latter is then oxidized. The carbon dioxide and oxygen values for the 50 grams of carbohydrate burnt as such are 73.3 grams and 53.3 grams respectively. The remaining 50 grams of glucose are isocaloric with 19.8 grams of fat. However, the carbon content of this fat is only 75.2 per cent of that contained in 50 grams of glucose. Since the conversion of carbohydrate to fat is an endothermic reaction, the remaining 24.8 per cent of the 50 grams of glucose must be oxidized to furnish the energy for the reaction. This latter oxidation yields 18.2 grams of carbon dioxide and requires 13.2 grams of oxygen. The oxidation of the 19.8 grams of fat yields 55.1 grams of carbon dioxide and requires 56.9 grams of oxygen. But, in the conversion of 37.6 grams of carbohydrate to the 19.8 grams of fat, 16.9 grams of oxygen are liberated. Hence

	CO <sub>2</sub> grams	O <sub>2</sub> grams
50 grams Glucose	73.3	53.3
19.8 grams Fat	55.1'	56.9
12.4 grams Carbohydrate	18.2	13.2
	<hr/>	<hr/>
	146.6	123.4
Oxygen liberated		16.9
		<hr/>
		106.5
CO <sub>2</sub> - O <sub>2</sub> = 40.1 grams		

Thus when the dietary carbohydrate is insufficient to furnish more than the total requirement of energy, and when it is impossible for the individual to store glycogen, it is legitimate to assume that the individual is oxidizing all of the ingested carbohydrate. In the unusual condition, when the individual is made to ingest carbohydrate whose calorific value is in excess of the total transformation of energy, special calculations will be needed.

Since it has now been shown that, under these conditions, all of the dietary carbohydrate is oxidized, the calculation proceeds as already described for submaintenance.

#### SUMMARY

(1) Eight individuals were fed, as nearly as possible, a maintenance diet while they continued to lead their usual lives.

(2) The twenty-four hourly insensible loss of weight was repeatedly determined and the average value for the insensible water calculated from it by subtracting the difference between the weights of the excreted carbon dioxide and the absorbed oxygen.

(3) It was shown that the heat removed by this water vapor was close to twenty-four per cent of the total heat production.

(4) A procedure was outlined for obtaining the carbon dioxide and oxygen values for any type of diet.

(5) Our results have shown that the determination of the twenty-four hourly insensible water furnishes an accurate method for calculating the heat production of individuals leading their routine lives.

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of Weight.



# AN IMPROVED METHOD FOR THE DETERMINATION OF WATER BALANCE<sup>1</sup>

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An earlier publication from this laboratory (1) dealt with the measurement of total water exchange. In the interval, the application of this method has given rise to a number of modifications which increase the accuracy of the results obtained, so that it appears advisable to discuss the subject again.

In a recent article (2), we have shown that the method of Benedict and Root (3) for the estimation of basal heat production from the insensible loss of weight, is subject to rather large errors. On the other hand, we have pointed out (4) that the determination of the amount of water vaporized in twenty-four hours gives an accurate method for estimating the total energy exchange in individuals under conditions of normal activity. This paper will deal with the water balance in one of these individuals on two different diets, since this data is well suited for the discussion of the principles involved. A portion of the data of this experiment is being reported in another publication (5).

The estimation of water balance necessitates the direct determination, or the calculation of four sources of water to the organism, and of three means of excretion of water from it. The factors involved in water balance may be advantageously listed as follows:

<i>Excretory water</i>	<i>Sources of water</i>
1 Water of urine	4 Water of food
2 Water of stool	5 Water drunk.
3 Insensible water	6 Water of oxidation
	7 Preformed water

<sup>1</sup> The expenses of this investigation were defrayed in part by a fund for the study of nutrition created by Mr. W. K. Kellogg, of the Kellogg Corn Flake Company Battle Creek Michigan.

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Let us first consider those factors which can be determined directly

1 For the determination of the water excreted in the urine, an aliquot of the specimen is placed in a tared weighing bottle, frozen and evaporated in a vacuum desiccator until it reaches constant weight. From the weight of the solids of the aliquot used, the total solids in the entire specimen can be calculated. The difference between the weight of the urine and the weight of the solids contained therein, represents the water thus excreted.

2 The water content of the stool can best be determined by evaporating the entire weighed specimen on a steam bath until its weight is constant, and the weight lost in this process represents the water content of this portion of the excreta.<sup>3</sup>

3 We have found it very convenient to place the subjects on a special diet, which, for short periods of observation, may be repeated each day, or, for longer periods, in order to avoid monotony, may consist of three twenty-four hour diets which are repeated in cycles of three days. In the latter case, the water balance is also determined in three day periods. A sample of the diet is prepared as nearly as possible like that served to the subject, dried and its dry weight determined. The water content of a diet depends considerably upon its preparation, but the dry weight varies only slightly from day to day. Accordingly, this value is subtracted from the total wet weight of the food for any period to determine the water of this portion of the ingesta.

4 The determination of the weight of the water drunk is a simple process. We have found it convenient to use a large thermos bottle fitted with a drinking tube. The bottle may be filled and weighed at the beginning of the period and weighed again at the end of the period. The difference in the weights represents the weight of the water drunk.

5 The determination of the weight of the water lost insensibly from the skin and lungs for any period, has been discussed at length in an earlier publication (4). We have found that the second approximation of the insensible water, obtained by the method described, is sufficiently accurate.

6 The determination of the water of oxidation depends on an accurate knowledge of the composition of the metabolic mixture. After

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<sup>3</sup> Such specimens lose an additional small but negligible amount of weight in the vacuum desiccator.

estimating the heat production from the insensible water, as described previously (4), the calculation of the metabolic mixture follows directly. The amount of protein burned may be calculated by multiplying the excretory nitrogen by 6.25. In the previous article (4), it was shown that, after a few days on any diet the carbohydrate of the diet is equal to the carbohydrate of the metabolic mixture. If we now subtract from the total heat production, the heat derived by the oxidation of protein and carbohydrate (i.e. 4 Calories per gram in each case), the difference is the heat derived from the oxidation of fat. This value divided by 9.54 will give the grams of fat oxidized. Thus we now have the three factors making up the metabolic mixture.

The water produced in the oxidation of this mixture is calculated by means of the following constants. Protein yields 0.41 gram of water for each gram oxidized, while 1.07 gram of water is derived from each gram of fat. The water of oxidation of the carbohydrate may be accurately obtained by estimating from the diet sheet, the amounts of glucose, sucrose and starch making up the total weight of the carbohydrate portion. The water produced by the oxidation of one gram of each of these carbohydrates is as follows: glucose, 0.60 gram; sucrose, 0.58 gram; and starch, 0.556 gram. The sum of the values obtained for protein, fat, and carbohydrates is the total water formed by the oxidation of the food in any period.

(7) The preformed water entering into the consideration of water balance is the amount of water accompanying any storage of tissue, or the amount of water previously held by any body tissue oxidized during the period. Since it is classified with the sources of water, it will be positive when there is a destruction of body tissue, or negative when there is a deposition of new body tissue. If neither of these processes obtains the preformed water is zero. Protein holds three times its weight of water, and fat holds water to the extent of about 10 per cent of its weight.

The deposition or destruction of body tissue is related to the energy balance. The extent of the storage of potential energy can be obtained of course, by comparing the total energy available to, and the transformation of energy of the subject over any period. A fairly accurate statement of the available energy may be obtained from the composition of the diet since protein and carbohydrate have 4 calories

available for each gram of footstuff, and fat, due to failure in absorption, yields about 9 calories for each gram ingested. When this sum is greater than the transformation of energy, there must be an accompanying storage of tissue, and the preformed water is accordingly negative. If protein is involved, the extent of its destruction or deposition can be obtained by comparing the protein intake with the oxidation of protein as represented by the excretory nitrogen. The energy accompanying this shift in body protein is 4 Calories per gram. The remainder of the energy deposited in, or obtained from the destruction of body tissue, must be due to the change in the amount of body fat, since carbohydrate balance was established prior to the experimental period. This value, divided by 9.54, gives the magnitude of the shift in the fat content. The weight of the fat and protein deposited or destroyed multiplied by 0.1 and 3.0 respectively, gives the amount of preformed water involved.

We have found that a more accurate knowledge of the metabolic mixture and the preformed water may be obtained by the use of the total heat values of the diet and the excreta. It must be remembered that when protein is oxidized in the bomb calorimeter, it yields 5.65 Calories per gram, and when oxidized in the animal body it yields only 4 Calories per gram due to incomplete oxidation. In the latter case, the unreleased energy is contained in the urine in the form of excretory products of protein metabolism. The oxidation of the average mixed fat in the calorimeter produces 9.54 Calories per gram of material. When the material is burned in the body it also yields 9.54 Calories. But in calculating the energy available from the fat ingested, the value usually used is 9 Calories per gram, to correct for the failure of the organism to completely absorb all of the fat ingested. On the other hand, an average mixture of carbohydrates, since they are completely absorbed and oxidized to carbon dioxide and water in the body, yield their full heat value (i.e. 4 Calories per gram) to the organism. If we now obtain the full heat value of the diet and stool by analyzing them in the bomb calorimeter, and calculate the energy content of the urine by multiplying its nitrogen by 8, the difference between the energy of the diet and the energy lost in the excreta, is the energy available to the organism. When the available energy is compared with the heat production, the amount of energy stored as tissue or liberated by destruction of tissue, is obtained.

It is next necessary to determine the weights of protein and fat involved. But this requires a prior knowledge of the composition of the diet. This may be obtained in the following manner. The dietary nitrogen multiplied by 6.25 gives the protein. The dried diet analyzed by the method of Soxhlet gives the fat. The total heat value (bomb calorimeter) of the diet less the energy contained in protein and fat (i.e. protein  $\times$  5.65 and fat  $\times$  9.54) gives the energy content of the carbohydrate portion. This value divided by 4 is the weight of carbohydrate in the diet.

Let us now assume that the available energy was greater than the heat production and that there was also a nitrogen retention. The nitrogen is presumably stored as protein, and the energy thus laid down is the nitrogen retention  $\times$  35.3 (i.e. 6.25  $\times$  5.65). The total energy stored (available energy - heat production) less the energy stored in the protein gives the energy deposited as fat. The weight of the fat is obtained by dividing this value by 9.54. The preformed water, in this case is a negative value, since tissue is deposited.

When energy is stored but body protein is destroyed, the total protein oxidized less dietary protein, gives the amount of body protein destroyed. This protein yields 4 Calories of energy and 3 grams of preformed water per gram of protein. The available energy here is the sum of the total energy of the diet and the total energy of body protein oxidized less the energy contained in the urine and stool. The energy stored is obtained by subtracting the heat production from the available energy. Since this is all stored as fat, the amount of fat deposited can be obtained by dividing this value by 9.54. The preformed water of the protein is in this case a positive value, and that of the fat is negative. The net preformed water is the algebraic sum of the two.

When the heat production is greater than the available energy, the calculation of preformed water involves the principles described above.

The water balance may now be obtained by supplying the numerical values to the water balance form on page 723. The total water in, less the excretory water, gives the water balance. If this difference is negative, there has been a loss of water previously held by the body, and if positive, it indicates a retention of water other than that stored with the tissue.

## DETERMINATION OF WATER BALANCE

TABLE I  
*Energy balance*

Date	Total energy of diet	Energy lost in urine and stool	Heat production Available (1-2)	Energy balance (3-4)*	Metabolic mixture				Protein balance				Fat balance				
					Calories	Calories	Calories	grams	Protein	Fat	Carbohydrate	grams	Calories	grams	Fat	Pre-formed water†	
November 5-7	9,445	742	8,703	8,657	46	270	490	726	1,049	0	0	0	0	46	5	-0.5	
8-10	9,445	700	8,745	10,012	-1,267	270	632	726	1,201	0	0	0	0	-1,267	-133	13	
11-13	9,445	603	8,842	7,853	987	270	406	726	959	0	0	0	0	987	+103	-10	
14-16	9,445	681	8,764	8,505	259	270	477	726	1,032	0	0	0	0	259	+27	-3	
17-19	9,445	657	8,788	9,252	-	464	270	552	726	1,116	0	0	0	-	464	-49	5
20-22	9,445	711	8,734	8,747	-	13	270	499	726	1,059	0	0	0	-	13	-1	0
23-25	15,017	716	14,301	8,788	5,513	187	314	1,261	1,132	50	282	-150	5,231	548	-55		
26-28	15,017	833	14,184	8,989	5,195	212	325	1,261	1,154	25	141	-75	5,054	530	-53		
29-De- cember 1	15,017	789	14,228	9,332	4,891	208	363	1,261	1,192	29	164	-87	4,727	496	-50		
2-4	15,017	679	14,338	10,137	4,201	193	453	1,261	1,284	44	249	-132	3,952	414	-41		
5-7	15,017	785	14,232	9,202	5,030	206	349	1,261	1,176	31	175	-93	4,855	509	-51		

Diets (grams) November 5-22 Protein 270, Fat 527, Carbohydrate 726 November 23-December 7 Protein 237, Fat 905, Carbohydrate 1,261

\* Positive values represent energy stored by the deposition of new body tissue, and negative values represent the energy released by the oxidation of body tissue

† A negative value for the preformed water indicates a subtraction of that amount from the available water, because body tissue in a corresponding amount has been deposited, and, similarly, a positive value means an addition to the available water because body tissue has been destroyed

The use of this method may be exemplified by means of the data obtained from a normal subject who first received a maintenance diet and then a supermaintenance diet. These data are presented in Tables I and II. Table I deals with energy balance for the periods. Table II contains a typical water balance sheet for each period.

In Table I the values expressed in columns 1 and 2 were obtained in the bomb calorimeter. The heat production was obtained from the insensible water by the method previously described (4). From November 5th to the 22nd the subject was in nitrogen equilibrium and consequently there was no shift in body protein. Hence all of the pre-formed water accompanied the shift in body fat.

TABLE II  
Water balance

Date	1	2	3	4	5	6	7	8	9
	Water of food and water drunk	Water of oxidation	Pre formed water*	Water in (1+2+3)	Water of urine	Water of stool	Inensible water	Water out (5+6+7)	Water balance (4-8)
November 5-7	grams	grams	grams	grams	grams	grams	grams	grams	grams
5661	1049	0	6710	2673	436	3582	6691	19	
8-10	5719	1201	13	6933	2354	444	4144	6942	- 9
11-13	5328	959	-10	6277	2276	364	3250	5890	387
14-16	5900	1032	- 3	6929	3123	393	3519	7035	-106
17-19	5394	1116	+ 5	6515	1948	446	3828	6222	293
20-22	5891	1059	0	6950	3446	447	3619	7512	-562
								Total	22
23-25	5858	1132	-205	6785	1588	447	3636	5671	1114
26-28	5752	1154	-128	6728	2689	518	3719	6926	-178
November 29									
December 1	5525	1192	-137	6580	2591	350	3863	6804	-224
2-4	5826	1284	-173	6937	2200	318	4194	6712	225
5-7	4925	1176	-144	5957	1750	420	3807	5977	- 20
								Total	897

\* A negative value represents water held with stored tissue.

During the first six days (2 periods) on the supermaintenance diet the subject added 936 grams of water to his body (November 23-28, Table II). He also stored glycogen with its preformed water. It is

impossible to determine how much glycogen was added, and consequently there is no way of knowing what portion of this 936 grams of new water was laid down as a normal constituent with the glycogen. This is an example of the way in which the method fails to give the desired information about water exchange when the body store of glycogen is changing. However, the subject could not have added more than 200 grams of glycogen, and this amount would require at most 600 grams of water, leaving 336 grams to be accounted for. This reasoning brings out the point that the subject during these six days has added water to his body in two different ways, since, after subtracting the maximum that can be included under the head of preformed water, there remains several hundred grams held in the body for some unknown reason. This latter increment seems to be a lasting addition since it did not come out in the next nine days. This might simply have been the amount of water held with the extra food in the gastrointestinal tract.

If we return now to the water balance obtained while the subject was on a maintenance diet (November 5-22, Table II), it will be seen that there was a noteworthy variation in the water content of the body from period to period in spite of the constancy of the diet. This shift cannot be attributed to changes in activity, as may be seen by comparing heat production and water balance. It emphasizes the lability of the mechanism which regulates the water content of the normal individual. For purposes of study it permits one to regard edema and its counterpart, dehydration, as disturbances of this mechanism brought about either by naturally occurring disease or by conditions set up by the investigator.

A common clinical method of studying the water exchange depends solely upon a comparison of the water drunk and the urine output. The values set forth in Table II show how erroneous such a consideration is. For instance, throughout the experiment the insensible loss of water through the skin and lungs was greater than the total water lost in the urine. The water of oxidation varied from 30 per cent to 75 per cent of the water eliminated in the urine. The average water drunk per period of three days was 3600 grams, and the average water in the urine was 2400 grams. A consideration of these two values alone would indicate a water retention of about 1200 grams per period, or a total

retention of 13 kilograms of water during the entire experiment. As is shown in Table II, the actual retention for the entire experiment was less than 1 kilogram. It is evident then that none of the factors involved in the water exchange can be omitted if an accurate knowledge of the water balance is to be obtained.

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- 5 Wiley, F H and Newburgh, L H, J Clin Invest, 1931 x, 733 The Doubtful Nature of "Luxuskonsumption"



## THE DOUBTFUL NATURE OF "LUXUSKONSUMPTION"<sup>1</sup>

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In 1911, and several times thereafter, Grafe and his associates (1) stated that the fasting, resting metabolism of an individual is not proportional to the surface area, but is significantly affected by the calorific value of the food previously ingested. They also postulated that the total metabolism is influenced in the same way by the total intake of energy. According to them, normal animals, including man, maintain a constant weight, almost without regard to the energy intake, and obesity is often nothing more than the failure of this alleged mechanism to respond normally to the stimulus of food. Finally, leanness is an over response to a normal stimulus.

They supported these views by the following type of experiments. A dog was starved for a long time, and its resting, fasting metabolism was then determined. This value was used as the basis for comparison. The dog was now offered an abundance of food, and gained weight rapidly. The metabolism was repeatedly determined in the fasting, resting state. The metabolic rate thus determined showed a progressive increase out of proportion to the increase in body surface. Table I sets forth their data in detail, rearranged by us.

This increase in metabolic rate of the overfed animal beyond that of the starved animal was accepted by them as proof that the resting, fasting metabolism of the normal animal is increased by previous overfeeding. But the metabolic rate obtained after prolonged starvation should not have been accepted as a proper basis for comparison, since Schöndorff (2) had previously shown that starvation caused a decrease

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TABLE I

*Effect of different degrees of nutrition upon basal metabolism, according to Grafe*

Diet and length of period	Body weight	Basal heat production per 24 hours		Basal metabolic rate	
		Determined	Predicted *	After Gräfe	Standard
Beginning	kgm 20 15	Calories 1056	Calories 987	per cent +54	per cent + 7
21 days Starvation 21st day	15 00	672	905	± 0	-26
7 days 2243 Calories per day 4th day	18 50	816	963	+19	-15
29 days 2580 Calories per day 29th day	20 25	1047	992	+53	+ 5
11 days 1659 Calories per day 11th day	20 17	1112	989	+62	+11
19 days 1120 Calories per day 19th day	20 00	1061	980	+55	+ 8
10 days 882 Calories per day 10th day	18 50	839	963	+22	-13
7 days Starvation 6th day	17 50	856	946	+25	- 9
2 months Unrestricted	21 00	1005	1005	+48	± 0

\* Predicted heat production was obtained from heat production determined after the animal had led an unrestricted life for two months. This value was corrected for changes in surface area as indicated by change in weight from 21 00 kgm.

in this rate Zuntz (3) later showed that this decrease might be as much as 30 per cent below the normal. In spite of the demonstration by Schöndorff, that a starved dog has a greatly depressed rate, Gräfe accepted the heat production of his dog, after 21 days' starvation, as a proper value for the basis of his calculations. The 24 hourly heat production of 672 Calories was thus erroneously used as the norm with which to compare the metabolism of overnutrition. When the animal was normal, in the usual sense of the word (i.e. after 2 months of food ad libitum), its heat production was 1,005 Calories. Starvation had accordingly reduced it 26 per cent. Liberal feeding permitted the basal metabolism to return to approximately the normal value, which at its height was 62 per cent above the starvation level but only 11 per cent more than the normal value.

In 1912, Grafe and Koch (4) reported the study of a man whose normal weight was 62 kgm and whose height was 156.2 cm. He was 35 years old. He came to the hospital suffering from stenosis of the pylorus accompanied by persistent vomiting. His weight had fallen to 40 kgm. His fasting, resting metabolism was determined, the stenosis was then relieved by operation, and ten days later a period of over-nutrition was begun. It lasted seven weeks, at the end of which time the patient's weight had again become 60 kgm.

Inspection of Table II makes it clear that the undernourished patient

TABLE II

*Effect of different degrees of nutrition upon basal metabolism according to Grafe*

Sex—M

Height—156.5 cm

Age—35 years

Condition of patient	Weight	Surface area	Calories per square meter per hour		Basal metabolic rate
			Determined	Predicted	
Before operation	kgm 40.0	square meters 1.34	30.5	39.0	per cent -22
	40.0	1.34	29.5	39.0	-24
After operation and period of overnutrition (100 Calories per kilo)	60.2	1.59	40.1	39.0	+ 2
	60.2	1.59	41.4	39.0	+ 5

exhibited the usual depression of the metabolic rate. When the operation had made it possible for him to absorb food in large quantities, the ingestion of 100 Calories per kilogram caused him to gain in the neighborhood of 22 kgm in less than two months, and in addition merely permitted the oxidative rate to return to the normal level. There is, then, no evidence that "Luxuskonsumption" increased the fasting, resting metabolism.

It should be recalled that Grafe had said that the normal animal maintained a constant weight in spite of overfeeding, because the extra food stimulated the total metabolism to such an extent that the excess was oxidized, but he published no data that dealt with the total metabolism.

We have, accordingly, reinvestigated this question. In a recent publication from this laboratory (5), we have shown that quantitative data regarding the total metabolism may now be secured, and such data are included in our study.

The subject was a very thin young man, who weighed 57.5 kgm (nude), and whose height was 180 cm (6 feet). He was 28 years old at the time the experiment was undertaken. At the age of 17 he had attained his full height and weighed only 50 kgm (110 lbs.). By the time he was 20 years old he had attained his present weight. During his twenty-first year, for a period of 3 months, he happened to be eating food that tasted unusually good. He was fully aware of eating much more food than usual, and feeling unusually indolent. During these 3 months his weight increased about 11 kgm (25 lbs.). Following this unusual experience, he again partook of food which made no unusual appeal to him. In spite of his unusual height and leanness, he was quickly satiated and did not indulge in the common custom of eating before retiring. He failed to derive the usual pleasure from sweets and pastries. His mother diligently tried, without success, to get him to eat what she considered a sufficient amount. She even forced him to take a variety of "tonics" with the hope of increasing his weight. Hence on the basis of his food habits, his leanness might as easily be attributed to an unusually small intake of energy as to an abnormally great oxidative rate.

On November 5, 1930, he began to eat all of his food in the hospital diet kitchen. His dietetic prescription was protein 91 grams, fat 186

grams and carbohydrate 241 grams, giving about 3,000 Calories. To avoid monotony the dietitian fed these materials in the form of different foodstuffs for three consecutive days and used precisely the same foodstuffs for every subsequent three day period. The occupation of the subject was that of a professional chemist, and he made no conscious alteration in the routine of his life. On November 23, 1930 the diet prescription was changed to protein 89 grams, fat 413 grams, and carbohydrate 445 grams, giving about 5,000 Calories. The food was fed according to the above described plan. During part of the second period his activity was slightly but definitely increased due to pressure of extra work. This second diet was continued through December 7, 1930. Thereafter, until January 5, 1931, he made a serious effort to continue to gain weight by overeating. From the latter date until March 19 he took food according to his desires.

Sample diets of each period covering the three day cycle, and intended to be identical with those served to the subject, were prepared by the dietitian except that the milk, cream, butter and sugar were omitted. The milk, cream and butter are regularly analyzed by the hospital chemist, and are found to conform with standard value. The sample diets were dried over a steam bath, weighed and ground to a fine powder. A thoroughly mixed sample was then ignited in a bomb calorimeter to obtain its heat value. In addition the nitrogen, fat and ash content were determined.

The nude, fasting weight of the subject was recorded every third morning. The total weight of the ingesta, and of urine and stool, was also obtained for each three day period. The urine and stools were analyzed for nitrogen and total solids, and the heat value of the dried stool was obtained by means of the bomb calorimeter. The basal metabolism was measured three times during the first period, five times during the second period, and several times thereafter.

The actual total energy of the diet for the eighteen day period was obtained as follows: the milk, cream, butter and sugar were found to contain, by calculation 33,246 Calories. The energy value of the remainder of the diet, as determined by means of the calorimeter, was 23,436 Calories, giving a total of 56,682 Calories. The dried stool contained 2,450 Calories, and the urine contained 1,644 Calories (urinary nitrogen  $\times$  8). The subtraction of the energy lost by excretion from

the total potential energy of the diet left 52,588 Calories available to the organism

The weights of the subject, ingesta, stool and urine, contained in Table III, permit the calculation of the insensible loss of weight. By

TABLE III  
*Data from our subject on maintenance diet*  
Diet—3,000 Calories

Date	Body weight at beginning of period	Weight of ingesta	Weight of urine and stool	Insen-sible loss of weight	Surface area	Basal metabolism		Basal metabolic rate	Respiratory quotient
						Pred- icted	Deter- mined		
Novem- ber 1930	grams	grams	grams	grams	square meters	Calories	Calories	per cent	
5-6-7	57,562	7,227	3,348	3,863	1 73	1,640	1,470	-10 4	854
8-9-10	57,578	7,285	3,031	4,413					
11-12-13	57,439	6,894	2,853	3,537	1 73	1,640	1,510	-8 0	811
14-15-16	57,943	7,466	3,750	3,801					
17-18-19	57,858	6,960	2,619	4,104	1 73	1,640	1,450	-11 5	776
20-21-22	58,122	7,457	4,132	3,899					
23	57,548							-10 0	
				Total 23,617			Average 1,475		

subtracting from the body weight at the end of the period the weight of the ingesta, and adding to this value the weights of the urine and stool, and subtracting this modified final weight from the initial weight, the insensible loss of weight was obtained (5). The total insensible loss for the eighteen days was 23,617 grams. In the earlier publication (5), we have shown that the total dissipation of heat may be calculated from the insensible water. Its weight in turn may be calculated by subtracting the difference between the weight of the exhaled carbon dioxide and that of the absorbed oxygen from the insensible loss of weight. A discussion of the method for determining these values may be found in the previous paper (5). In this instance they were 17,674 grams of carbon dioxide and 16,021 grams of oxygen, giving a difference of 1,653 grams. Thus the insensible water was 21,964 grams. The heat re-

moved by it was obtained by multiplying it by 0.58. This heat represents 24 per cent of the total heat dissipated (5). The heat dissipation calculated in this manner was 53,087 Calories for eighteen days, or 2,947 Calories per twenty-four hours. Accordingly, it is true that this subject, leading his usual life, transformed energy at the rate of 2,947 Calories per day when he was receiving 2,922 Calories per day.

Will he, then, transform more energy merely because he ingests more food?

The subject was, accordingly, fed the second diet, the calorific value of which was roughly 5,000 Calories per day, for 15 days. Accurate information was obtained for this period in the same manner as has been described for the first period. It was found that the total energy of the diet was 75,125 Calories for the period. The stools for the period contained 2,745 Calories, and the urine contained 1,056 Calories, giving a total of 71,324 Calories available to the organism.

TABLE IV  
*Data from same subject on supermaintenance diet*  
Diet—5,000 Calories

Date	Body weight at beginning of period	Weight of ingesta	Weight of urine and stool	Insensible loss of weight	Surface area	Basal metabolism		Basal metabolic rate per cent	Respiratory quotient
	grams	grams	grams	grams	square meters	Calories	Calories		
<i>November 1930</i>									
23-24-25	57,548	8,324	2,258	4,143					
26-27-28	59,471	8,218	3,463	4,227	1.76	1,651	1,560	-5.5	869
29-30-									
Decem ber 1	59,999	7,991	3,189	4,369	1.77	1,660	1,545	-6.9	875
2-3-4	60,432	8,292	2,739	4,691	1.77	1,660	1,575	-5.1	843
5-6-7	61,294	7,391	2,414	4,313	1.78	1,670	1,600	-4.2	903
8	61,958				1.79	1,679	1,610	-4.1	.880
				Total 21,743					

Table IV gives the weights of the subject, ingesta, stool and urine for the second period. The total insensible loss calculated from the

table was 21,763 grams. The total nitrogen of the urine was 132 grams. The metabolic mixture was calculated from the carbohydrate of the diet, the nitrogen excretion and the insensible loss of weight by the method described in the earlier paper (5). It was found to consist of 871 grams of protein, 1,928 grams of fat, and 6,315 grams of carbohydrate for the period of fifteen days. The corresponding carbon dioxide and oxygen values were respectively 16,357 grams and 13,752 grams. The difference, 2,605, subtracted from the total insensible loss, 21,763, gave 19,158 grams of insensible water for the period. The total heat dissipation indicated by this value was 46,298 Calories for fifteen days, or 3,082 Calories per day. Accordingly, the subject transformed 135 more Calories of energy per day when he was ingesting about 5,000 Calories than when the intake of energy was about 3,000 Calories, an increase of about 4.5 per cent in total metabolism.

The average surface area for the second period was 2.3 per cent greater than for the first period. Other things being equal, this would call for an average increase of 67.5 Calories per day in the second period, leaving 68 Calories per day thus far unaccounted for. These 68 Calories may be properly attributed to the specific dynamic effect of the extra fat and carbohydrate of the diet in this period, since there is a quantitative relationship between the amount of material ingested and its dynamic effect. Lusk (6) has recently stated that the metabolism is increased after the ingestion of fat and carbohydrate by four per cent and six per cent respectively of the Calories furnished by these foodstuffs. In the second period the fat was 116 grams and the carbohydrate 180 grams greater per day than in the first period. Four per cent of the extra Calories in the fat is 42, and six per cent of the extra Calories in carbohydrate is 43. Thus the total metabolism should have increased in the second period 85 Calories, due solely to the extra food ingested. It will be recalled that the calculations required us to account for 68 Calories in this way. Thus all of the increase in the total metabolism is disposed of without recourse to “Luxuskonsumption.”

From Tables III and IV it may be seen that the basal metabolic rate in the second period was about 5 per cent higher than in the first period.

An inspection of the respiratory quotients for each period shows that they were markedly higher when the subject was taking the excessive

TABLE V  
*Return of subject to maintenance diet*

Date	Diet	Body weight at end of period	Surface area	Basal metabolism		Basal metabolic rate per cent	Respiratory quotient
				Predicted	Determined		
December 8-15	Deliberate intake of food greater than desire	62.610	1.79	1,679	1,580	-6.1	842
December 15-22	Same as above	62.984	1.80	1,688	1,490	-11.7	857
December 22-January 13	Food according to desire	62.256	1.79	1,679	1,490	-11.3	765
January 13-February 13	Same as above	61.574					
February 13-March 19	Same as above	60.943					

diet. It is generally conceded that this phenomenon indicates that the disposal of the previously ingested food is still going on in other words, that the metabolism has not yet fallen to its basal level because of the continued specific dynamic action.

In the third period when the subject was still over eating, as evidenced by continued gain in weight, the basal metabolism quickly fell to its original level. This was to have been expected since the diet was now only moderately excessive and its specific dynamic action was complete before the determination of the basal metabolism on the following morning.

Here again there is no need of postulating a "Luxuskonsumption" to account for the facts.

Further information in regard to the response of the subject may be obtained from an analysis of the gain in weight during this period. The actual addition of body weight was 4,410 grams. It is conceivable that all of it might have been due to the retention of that amount of water. If this were true, it would necessarily also be true that he had burned all of the diet. However, an analysis of the data gives a quite

different answer (7) The comparison between the available energy of the diet and the transformation of energy shows that the subject acquired 24,830 Calories deposited as 179 grams of protein and 2,499 grams of fat These materials in the form of body tissue would deposit with them 787 grams of water Hence, the addition of tissue amounted to 3,465 grams In addition to this weight there should have been a water retention of 945 grams to account for the total gain in weight The determination of the water exchange for the period accounted for the retention of 897 in addition to that deposited with the protein and fat These considerations offer further evidence to demonstrate that the extra energy absorbed from the excessive diet was, in the main, stored and not burned

#### DISCUSSION

About 20 years ago, Grafe stated that the oxidative rate in the animal organism was significantly affected by the amount of energy taken in It had already been clearly demonstrated by several different investigators that the heat production in the basal state falls as much as 30 per cent due to prolonged starvation Much later, F G Benedict (8) fed a group of men, who were habitually ingesting 3,200 to 3,600 Calories, a diet containing 1,400 Calories After three weeks the average weight of the subjects had declined 12 per cent and their basal metabolism had fallen 18 per cent They were now able to maintain this new low weight on 1,950 Calories, and the basal metabolism remained low It is true then that the organism can reduce its rate of oxidation in response to underfeeding and, if the latter is not too extreme, weight may be maintained after an initial loss This is clearly an adaptation capable of prolonging the life of the organism in the face of famine On the other hand, it is not easy to picture any advantage obtainable through the ability to dispose of an over abundance of food rather than to store it so that it would be available in time of need

Grafe, however, tried to prove that the normal mammalian organism did automatically dispose of excessive food through the mechanism of an increased metabolic rate We have shown above that his data do not support his hypothesis, for when the fasting, resting metabolic rate of his dogs obtained during a period of over nutrition is compared with this rate obtained when the dog was apparently normal, no signifi-

cant increase is found. He obtained an apparent increase by first pushing the rate down by means of a long period of starvation. He thus obtained a metabolic rate 26 per cent lower than the normal but, nevertheless, he accepted this value as a proper basis for comparison. The same criticism applies to the study of the patient whose rate was 22 per cent below normal when he was emaciated and in whom very marked "Luxuskonsumption" permitted the rate to return to the usual level for such an individual in health.

Even though the fasting, resting metabolism does not appear to be stimulated by previous over nutrition, it may still be true that the total transformation of energy can be increased by this factor. Grafe did not deal with this phase of the subject at all. However, due to the development of a method in this laboratory, such data are now obtainable without the use of a calorimeter.

The subject whom we studied belonged to the group of persons alleged to be pathologically lean because, accepting the dictum of Grafe, they oxidize all of the food taken into the body without regard to quantity. The slight increase observed in both the basal and total metabolism, on a super-maintenance diet, was found to be entirely attributable to the increase in surface area plus the extra specific dynamic effect of the greater diet. It was equally clear that this subject was quite capable of gaining weight when he took food in excess of his habitual desire. A scrutiny of his life brought out the important fact that he had always been indifferent in regard to food, and that he had consequently eaten sparingly. However, during a short period of his life he had food set before him which was so attractive that it overcame his indifference and he gained a large amount of weight during this interval. A recognition of the fact that he habitually ate less than the usual amount of food for his group is sufficient reason for his thinness.

Clearly the body weight is affected on the one hand by the individual metabolic requirement and on the other hand by the total intake of energy. Evidently the mechanism commonly called appetite, functions to maintain a balance between the supply and the demand. This is hardly the place to discuss the well known factors that influence appetite and individual metabolic requirements. Observations and special studies on appetite (9) in this clinic have convinced us that the

abnormalities of body weight are regularly due to a failure of the appetite to make a complete adjustment between the inflow and outflow of energy

#### SUMMARY

(1) The basal metabolism and the total transformation of energy of an unusually thin subject were recorded when (1) he was on a maintenance diet and (2) when he was being vigorously overfed

(2) It could not be shown that either the basal or the total transformation of energy per square meter of body surface was increased by more than the increment due to the extra specific dynamic action of the additional food

(3) On the other hand, the subject added about 4.5 kilograms to his weight in 15 days when he was being overfed

(4) No support for the "Luxuskonsumption" hypothesis of Grafe was secured

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## THE DETERMINATION OF POTASSIUM IN CARDIAC MUSCLE AND THE PRESUMABLE INFLUENCE OF THE BETA RADIATIONS ON THE RHYTHM

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A summary of a fairly large series of analyses on cardiac muscle for the principal inorganic constituents was published some time ago (Scott (1)) These determinations included both potassium and sodium and the method seemed sufficiently convenient and accurate at the time. However, it occurred to the author that at least for potassium, the function of which appears to be both complex and important the procedure might be simplified somewhat, thus enabling one to obtain even more reliable data, and finally to apply the results so obtained to the calculation of the kinetic energy developed by the emanation of the active Beta particles

For this reason a second series of determinations of potassium on thirty hearts was carried out and the work was simplified by the omission of steps unavoidable in the first This was conducive to greater accuracy and it is believed that the results are as close an approximation to the true potassium content as it is now possible to obtain

A certain amount of controversy has arisen over the advisability of dry ashing organic material containing alkali salts However, Raab (2) used dry ashing and Egg and Klinke (3) employed Stolte's method with good results Constantino (5) used a modification of the dry incineration method which in the end involved heating to drive off sulphuric acid Ernst and Scheffer (6) showed conclusively not only by incineration of dried muscle but also by exposure of potassium salts alone to long periods of heating that the objections of other observers are not tenable, and their tables indicate that no appreciable losses occur even after six days in an electric furnace

This is in a measure confirmed by the findings with 6.1621 grams KCl. The salt which had been dried was heated in a palaurium dish for three and one half hours to a faint red heat insufficient to melt it completely, weighings being made at intervals of 1 hour, 20 minutes, 1 hour, and 1 hour and 10 minutes. The losses which may be attributed to water held tenaciously by the crystalline salt were respectively 0.0023, 0.0028, and 0.0062 grams. This was followed by a period of heating for six hours with three weighings at 1 hour and 10 minute intervals and two more at 1 hour and 15 minute intervals. The results were, in order of the weighings, -0.0002, +0.0004, -0.0002, +0.0001 and +0.0001 gram, that is, there was virtually no loss whatever. The increases in weight may have been due to moisture absorption while weighing. When, however, the heat was increased and the salt kept in a fluid condition for 1 hour and 5 minutes the loss was 0.1693 gram and white fumes were given off. This degree of heat was never applied to the incineration of the tissue, and is moreover not necessary. The latter process required as a rule two and one half to three hours and the silica dish in which it was carried out never showed more than a faint red, nor was the ash ever allowed to melt.

Duplicates made by myself on cardiac tissue heated three to four hours agree extremely well in the ash content. Obviously there is small chance of error from this source.

#### METHOD

The choice of the method for estimating the potassium was decided upon after careful review of the literature and insofar as accuracy is concerned the old chloroplatinate precipitation method does not appear to have been surpassed.

No moisture determinations were made. Sixty grams of fresh ground heart muscle were dried thoroughly in a silica dish and the contents ashed at a very faint red heat. The temperature was kept low in order to avoid melting the ash in which case it is sometimes impossible to get rid of all the carbon and the ash is not always white. Some ashes resist heating for periods longer than it is desirable to keep the dish over the flame. For this reason the ash percentages are only approximately correct in the second and third decimal places.

When incineration was complete and after weighing, the ash was

treated with strong HCl and evaporated to dryness, it was again heated to dehydrate silicic acid, redissolved with a few drops of HCl and carefully transferred to a 150 cc beaker. Strong ammonia was added and the solution stirred thoroughly, scraping the sides of the beaker with the rod. This precaution facilitates the separation of the magnesium. Clear lime water from C P CaO was added to get rid of most of the phosphates and finally slight excess of saturated ammonium oxalate solution to precipitate the lime. No attempt was made to free the solution completely of phosphates, nor is it necessary. Ferric phosphate, however, is troublesome and it passes the filter in small amounts despite the use of wash water containing ammonium hydroxide and ammonium acetate. As will be observed, this possible chance for error is taken care of later.

After standing over night the precipitate is compact and may be readily removed by filtration. The filtrate was diluted to 500 cc in a calibrated measuring flask to which a two drop 50 cc. pipette had been adapted.

For each determination 50 cc equivalent to 6 grams of heart muscle were measured into a beaker and evaporated carefully to dryness on a hot plate. It is advisable to acidify with HCl after the free ammonia has been driven off to minimize the danger of spattering. The ammonia salts were then expelled, the residue taken up with 2 drops of concentrated HCl and 5 cc. of water. Platinum chloride (approximately 10 per cent) in excess was added, and the contents evaporated slowly on a water bath, care being taken not to place the beakers in contact with the steam. Complete dryness was however avoided. In the latter event, one or two drops of HCl and a few cubic centimeters of water were added, and the evaporation repeated.

When the proper condition had been attained, that is with the residue still moist, the beaker was removed, cooled and diluted with 1-2 cc of water and 95 per cent alcohol added in small quantities. The first addition should be about 5-10 cc in which the crystals of chloroplatinate are broken and ground up with a stirring rod provided with a flattened end. Afterward the remainder of the alcohol up to 50 cc. may be added, the whole stirred thoroughly and allowed to stand 3-4 hours or preferably over night.

The potassium chloroplatinate was filtered off in Gooch crucibles

having mats made from asbestos that had been thoroughly digested with strong HCl and washed. The reduction and subsequent manipulations are otherwise the usual ones given for this method. The weight of reduced Pt  $\times 0.3993 = K$  in grams.

One point, however, deviates somewhat from the routine. This consists in moistening the reduced platinum with a few drops of strong HCl *after* removing the soluble salts with hot water and again washing with hot water. By this precaution and by dissolving the alkali residue in the beaker with HCl before precipitation with platinic chloride solution and furthermore by keeping the chloroplatinate precipitate faintly acid the error due to ferric phosphate may be avoided. Sodium phosphate does not influence the precipitation of potassium, and lime and magnesium are eliminated, so that the only sources of error aside from those defects inherent in the method itself appear to be taken care of fairly well. The faint excess of HCl does not seem to increase the solubility of potassium chloroplatinate in the alcohol. Neubauer (7) showed that quite appreciable quantities of sulphuric acid did not vitiate the end results.

#### Checks

A number of the hearts were assayed in duplicate. That is, two 60 gram portions were taken. Usually but not always these checked very closely with one another. As a rule, however, three to six 50 cc portions of the diluted ash solution each representing 6 grams of the original heart were used, and there is no reason to think that duplicates were necessary.

These determinations always agreed to the second place and no series is included that did not check in at least three and ordinarily five determinations within the third decimal place. When three very close checks were obtained no further determination was made. As examples, the following may be cited. Heart number 128 showed 0.178, 0.182, 0.178, 0.173 per cent K while heart number 140 which is not so close gave the following figures 0.319, 0.317, 0.329, 0.324, 0.320 per cent K.

Altogether 120 determinations of K were made on thirty hearts and of these, eight have five checks which were used in estimating the probable error of the individual determination. This was obtained by using the formula  $\pm 0.6745 S$  where S is the standard deviation for a

series of checks. The probable errors were respectively  $\pm 0.0018$ ,  $\pm 0.0014$ ,  $\pm 0.0025$ ,  $\pm 0.0012$ ,  $\pm 0.0027$ ,  $\pm 0.0027$ ,  $\pm 0.0021$  yielding a mean P E for individual determinations of  $\pm 0.0021$  gram K per 100 grams.

An additional check on the method consisted in determining the K in a mixture of disodium phosphate and potassium chloride approximating conditions in the heart sample. The results are expressed in Table 1. They furnish, I think, a fair demonstration that phosphates do not influence the accurate determination of K in mixtures.

TABLE 1

*Determination of potassium in the presence of phosphates*

0.2500 KCl and 0.5000 gram Na<sub>2</sub>HPO<sub>4</sub> 12 H<sub>2</sub>O to 500 cc.  
50 cc. equivalent to 0.0250 KCl and 0.0500 Na<sub>2</sub>HPO<sub>4</sub> 12 H<sub>2</sub>O per determination

	Pt found grams	KCl grams
1	0.0338	0.0257
2	0.0336	0.0256
3	0.0334	0.0254
4	0.0334	0.0254
5	0.0330	0.0250
6	0.0330	0.0250
7	0.0334	0.0254
Mean	0.0333	0.0254

(The KCl used was B and A.C.P. salt. The Na<sub>2</sub>HPO<sub>4</sub> was marked C.P. but the origin is unknown. One gram yielded 0.0074 gram Pt = 0.00028 gram KCl for 0.0500 gram Na<sub>2</sub>HPO<sub>4</sub> 12 H<sub>2</sub>O.)

A summary of the data derived from the analyses including the probable error and standard deviation is shown in Table 2. The probable error of the mean is determined from the formula  $\pm \frac{0.6745 S}{\sqrt{N}}$

in which S is the standard deviation and N the number of observations. There is considerable variation in the means of the individual deter-

TABLE 2  
*Summary of analyses on moist tissue of 30 hearts*

	Ash grams per 100 grams moist	Potassium grams per 100 grams moist
Mean	0.976	0.231
Probable error of mean	$\pm 0.0157$	$\pm 0.0066$
Standard deviation	0.1277	0.0538

minations For example the ash varied from a minimum of 0.691 per cent to a maximum of 1.290 per cent, the potassium from a minimum of 0.129 per cent to a maximum of 0.316 per cent The lower figures for ash and K were obtained on a small, soft, flabby, thin walled and obviously much degenerated heart from a man 69 years old, while the highest were also from a relatively small but thick walled male heart

In general it cannot be said that either pathological changes, age of patient, illness that was responsible for death or any other factor is in any way correlated with the potassium content This observation with reference to other inorganic constituents has already been noted (Scott (1))

#### *Edema and potassium content of heart muscle*

Among the hearts in this series there were seven from patients showing unmistakable signs of cardiac failure with edema There were probably more but nothing definite can be obtained from the records The potassium content of these hearts is compared with that of an equal number from patients dying of diseases in which edema did not occur The latter comprised three from tuberculous patients and one each from victims of bronchopneumonia, epithelioma, duodenal ulcer, and lues with resultant complications

The mean of the potassium in hearts from the seven patients with edema was 0.220 per cent, and from the seven non-edematous cases 0.218 per cent This agrees approximately with a series of determinations of potassium and sodium on fifteen hearts from edematous patients and a like number from non-edematous cases previously reported by Scott (8) The present analyses are believed to be more accurate but are merely confirmatory In neither series is there evidence that the myocardium contains more or less potassium when the individual dies of cardiorenal failure with edema than when he succumbs to another disease not productive of edema This is not in agreement with the observation of Harrison, Pilcher, and Ewing (9), who concluded that with chronic congestive heart failure the cardiac muscle contained less potassium than in other diseases producing death

#### *Possible effects of β-radiations of potassium on the heart muscle*

In 1908 Campbell and Wood (10), Campbell (11, 12) alone and McLennan and Kennedy (13) demonstrated beyond a doubt that the

potassium atom is continuously disintegrating with the emanation of Beta particles and the concensus of opinion seems to be that with the exception of very minute quantities of radium it is the only radioactive element in the human body.

On the basis of these and other observations and by comparing the activity of potassium with that of uranium, rubidium and radium, Zwaardemaker (14-15) estimated the kinetic energy produced by the Beta particles of K in the human heart weighing 300 grams as  $4.1 \times 10^{-4}$  ergs per second.

That this estimate is far too high can readily be shown when we consider the recent investigations of Mühlhoff (16), who also exhaustively cites prior contributions on the subject. Furthermore, Zwaardemaker assumed that the Beta particles of potassium have an average velocity approximating 66 per cent that of light, whereas Kovarik (17) states that absorption coefficients indicate the velocity to be between 82 and 83 per cent.

Besides Beta particles the potassium atom also produces Gamma radiations. These have been definitely established by Mühlhoff (16), Kohlhorster (18) and Behounek (19). Unfortunately the evaluation of Gamma rays in terms of kinetic energy is not feasible and at present only the estimation of the kinetic energy developed by the Beta particles is possible.

By employing the absolute number of 23 Beta emanations per second per gram of the element potassium as given by Mühlhoff, and taking the average velocity to be 82 per cent that of light, one can readily calculate the kinetic energy developed by one milligram of K.

The energy of a Beta particle with a velocity of  $v = \beta c$  is

$$E = m_0 c^2 [(1 - \beta^2)^{-1/2} - 1] \quad (\text{Kovarik and McKeehan (20)}),$$

in which  $m_0$  the mass of a slowly moving electron, is  $9 \times 10^{-28}$  grams,  $c$  the velocity of light,  $2.9986 \times 10^{10}$  cm per second, and  $\beta$  the ratio of the velocity of the Beta particle to the velocity of light, in this instance 0.82. The kinetic energy developed by 0.001 gram K is thus shown to be  $1.392 \times 10^{-8}$  ergs per second.

Now assuming that the mean percentage of potassium in the heart muscle is approximately 0.231 per cent, then the disintegration of the atoms of this element will develop  $9.64 \times 10^{-6}$  ergs per second in a heart containing 300 grams of muscular fibers.

This amount of energy may appear to be extremely minute but it is hundreds times greater than the minimum necessary to stimulate either the optic or the auditory nerve endings and moreover it is continuous from birth to death, varying only in degree.

The experimental work of Zwaardemaker has shown that other radioactive and, insofar as tissue is concerned, chemically inert substances are interchangeable with potassium, which makes it evident that the radioactive character of potassium exerts a tremendously important influence on rhythmicity. One may readily conceive that the free energy of the Beta particles can be cumulative and reaching a maximum transform the potential energy of the heart muscle in response to node and bundle impulses into the enormously greater manifestation of kinetic energy, the systolic contraction. This would constitute the function of potassium in the heart muscle in some respects analogous to that of the cap on a high explosive charge, either directly or indirectly as a detonator.

Such a conception of the rôle of potassium taken in conjunction with Howell and Duke's (21) observations that vagus irritation increases potassium elimination might afford a rational picture of the phenomenon of ectopic beats as well as of other disturbances of cardiac rhythm.

#### SUMMARY AND CONCLUSIONS

1 It is believed that the method employed for the determination of potassium in the human heart muscle yields quite accurate results.

2 The mean percentage of potassium expressed as the element K was found to be 0.231 per cent.

3 There is no material difference between the content of potassium in hearts from edematous and non-edematous cases.

Acknowledgment is herewith gratefully accorded Prof D S Elliot of Tulane and Prof A F Kovarik of Yale University for their kindly suggestions.

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## GASTRIC SECRETION AFTER HISTAMINE ITS EVALUATION AS A QUANTITATIVE TEST IN DIGESTIVE DISORDERS

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### INTRODUCTION

With the development of a simple technic for fractional gastric analysis by injecting histamine subcutaneously as a stimulant for stomach secretion, we adopted this method as a routine procedure for it seemed to offer advantages over the gruel meal because it eliminated the factors of dilution and buffering, and also because it afforded a method of determining more accurately the volume of juice secreted by the stomach. In time, however, because of the wide variations in the height of the acidity curves, obtained irrespective of the ultimate clinical diagnoses, we began to doubt its value. We have undertaken therefore, to compare the results with histamine with those obtained after the gruel meal and after Liebig's extract.

Few studies have been reported of gastric secretion of normal persons after the injection of histamine. Schiff (1) found that ' of 33 normal males 26 showed acidities of 120 cc. N/10 per 100 cc or less, 7 greater than 120 of 33 normal females 24 showed acidities of 120 or less 9 over 120 ' These figures give little idea of the distribution of his figures for the acidity but from the spot diagram of highest total acidity attained in a recent series of normals, reported by Pollard and Bloomfield (2), it is evident that the figures are rather evenly distributed between 40 and 150

### METHOD OF STUDY

The first method of study was to compare the results obtained by submitting 130 patients to the histamine test (*a*) with those secured by

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TABLE 1  
*Diagnoses of patients submitted to histamine test*

Diagnosis	Number of cases
Chronic gastritis	5
Carcinoma of stomach	3
Gastric polyposis and hypertrophied mucous membrane	2
Functional gastric disturbance (secretory or motor)	6
Gastric neurosis	1
Bezoar	1
Duodenitis	8
Duodenal ulcer	16
Duodenal ulcer with perforation (postoperative)	2
Duodenal ulcer, jejunal ulcer postoperatively	2
Duodenal ulcer, gastric ulcer (postoperative)	1
Duodenal ulcer postoperative	1
Duodenal ulcer, chronic cholecystitis	1
Duodenal stasis	6
Duodenal adhesions (periduodenitis)	8
Colitis	5
Colitis, mucous	2
Colitis, ulcerative	2
Diverticulitis of colon	1
Small intestinal stasis	1
Visceroptosis	4
Constipation	3
Intestinal adhesions	3
Congenital veil of intestine	3
Chronic cholecystitis	17
Cholecystectomy	7
Carcinoma of pancreas	1
Esophageal spasm	2
Neurasthenia	8
Menopausal neurosis	3
Hypertension	2
Sacro-iliac disease	1
Syphilis	1
Hypothyroid and hypopituitary function	1
Total	130

us on a group of patients in the same age decade and with the same diagnoses tested by the gruel meal, and (b) with those secured by Farr (3) on a group of patients whose secretion was stimulated by Liebig's beef extract. The diagnosis of the 130 patients undergoing the histamine test are listed in Table 1. Farr's patients had various psychoses but were in good physical condition.

The second method of study was to compare the results obtained by performing both the histamine and the oatmeal gruel tests on a group of 39 patients. Each test was made one or more times. The diagnoses of this group are listed in Table 2.

TABLE 2

*Diagnoses of patients submitted to both histamine test and fractional test meal*

Diagnosis	Number of cases
Chronic gastritis	2
Polyposis of stomach and hypertrophied mucous membrane	2
Gastric neurosis	1
Duodenitis	5
Duodenal ulcer	4
Duodenal diverticula	1
Duodenal stasis	1
Colitis	2
Carcinoma of colon	1
Diarrhea	1
Constipation	4
Congenital veil of intestine	2
Chronic Cholecystitis	3
Neurasthenia	
Addison's anemia	2
Diabetes	1
Arthritis Neisserian	2
Pulmonary tuberculosis	3
Unclassified	1
Total	39

All patients had been thoroughly studied by radiological investigation, and in some instances the diagnosis was established at operation.

*Technic of histamine test*

After an all-night fast, the fasting gastric contents were removed, as completely as possible, through a duodenal tube passed into the stomach, this specimen was discarded. Juice secreted during the next two 15-minute periods was allowed to syphon off in order to establish the rate of secretion before stimulation; these specimens were labelled "fasting content." Histamine (ergamine acid-phosphate 1:1000 solution) 0.3 cc was then administered hypodermically. The resulting juice was collected by syphonage for one hour or more, receptacles were changed at 15 minute intervals. The volume was measured, and the acidity was determined by titration with N/50 NaOH using Topfer's reagent and phenolphthalein as indicators. The acidity was expressed in the usual manner of cc N/10 acid per 100 cc of gastric juice.

The juice came readily, once syphonage was started, rarely was there any difficulty from clogging of the tube. Satisfactory specimens were usually obtained, except in a few cases of achlorhydria in which secretion was sparse. Patients were instructed to expectorate all saliva during the test.

*Technic of Liebig's extract test*

The fasting stomach was emptied by means of a duodenal tube, and preliminary samples of contents were aspirated at 5 minute intervals for 15 minutes. One gram of Liebig's extract in 50 cc of warm water was introduced through the tube into the stomach, and allowed to remain for 5 minutes, the stomach contents were then withdrawn as completely as possible. Subsequent samples were collected by continuous aspiration during 15 minute intervals for one hour. Practically pure stomach juice was thus obtained.

*Technic of gruel test meal*

The fasting gastric content was removed through a duodenal tube after the patient had fasted overnight. Three hundred cc of oatmeal gruel, uniformly prepared and strained through gauze, was swallowed while the tube was in place. The first 10 cc sample of gastric juice was taken at the end of 30 minutes. Following this, samples of 10 cc were extracted at 15 minute intervals for a total of an hour and a half. The residue was then removed and its volume determined. The acidity was determined as described above.

## RESULTS

*Acidity*

The values for maximal free acid attained in each test after histamine for the whole group, excluding those cases diagnosed as duodenal ulcer or duodenitis, are presented in Fig 1. The results in patients with ulcer and duodenitis are similarly illustrated in Fig 2. Farr's data obtained by the use of Liebig's extract, are given in Fig 3. In Fig 4 the results obtained after oatmeal gruel are given for a group of patients whose condition was similar to that of the group shown in Fig 1.

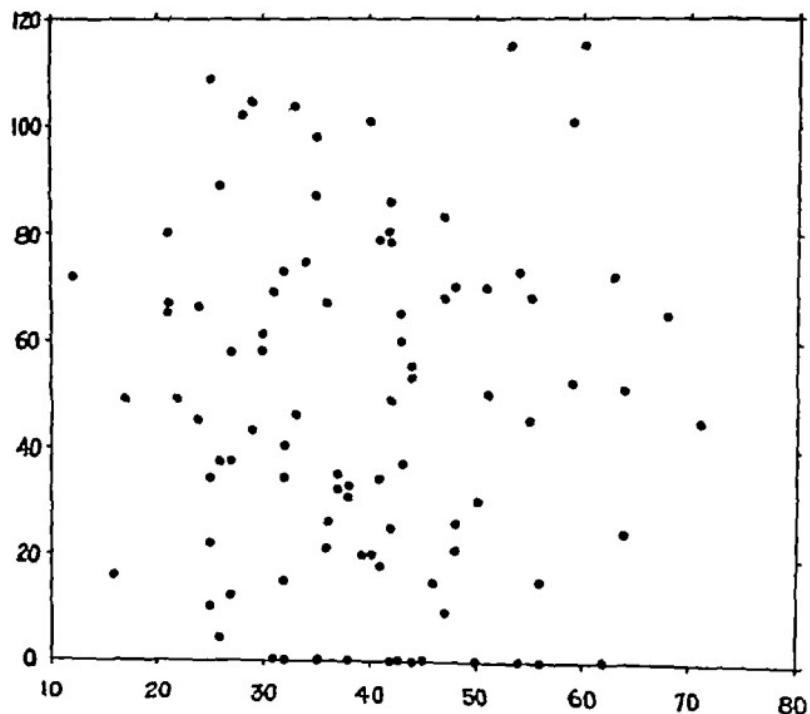


FIG 1 RESULTS OF HISTAMINE TEST ON WHOLE GROUP EXCLUSIVE OF CASES WITH DUODENAL ULCER AND DUODENITIS  
Age plotted as abscissae against highest free acid as ordinates

From an examination of the results of the histamine test it is clear that the maximal acidity that was attained varied rather widely in its distribution, and did not tend to fall about the mean value. This same

scattering of figures occurred with Liebig's extract, no correlation of these variations with the various psychoses or moods of these patients has been recognized. The observations made after oatmeal gruel on the same type of patient as that studied in the histamine test showed greater constancy in the maximal acidity.

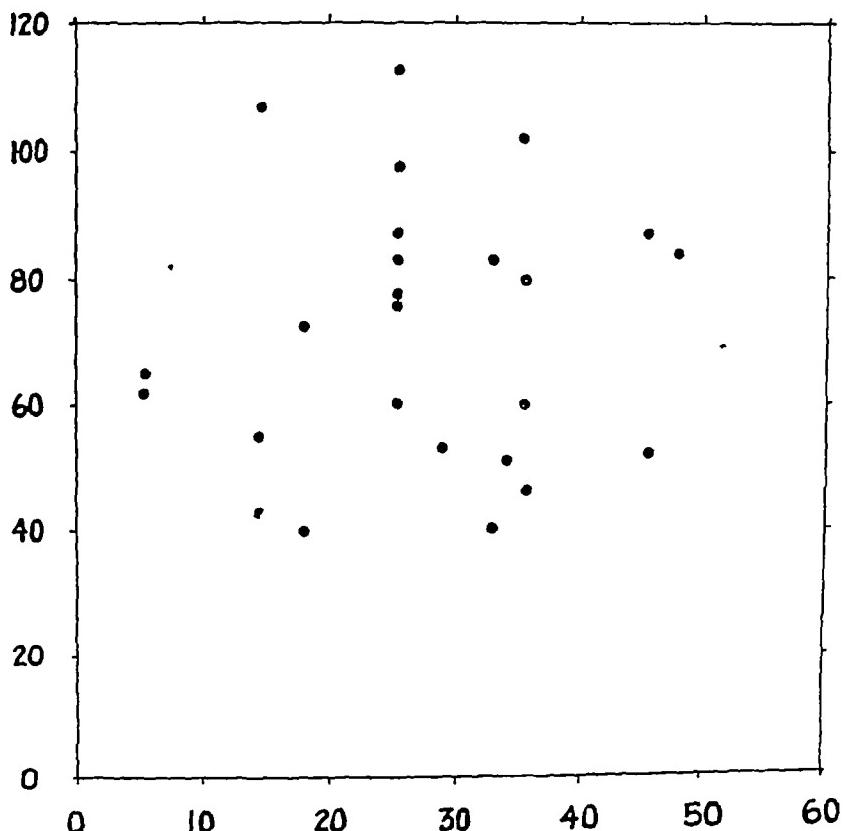


FIG. 2 RESULTS OF THE HISTAMINE TEST ON CASES WITH DUODENAL ULCER AND DUODENITIS

Age plotted as abscissae against highest free acid as ordinates

The results of studying the response to both histamine and oatmeal gruel in the same patients are illustrated in Fig. 5. There is no correlation between the acidity attained after oatmeal gruel and after histamine, nor could the variations after histamine stimulation be correlated with the diagnoses, except in the instance of the achlorhydrias.

We thus find very variable maximal acidities following stimulation by either histamine or Liebig's extract after both of these substances the stomach secretes into a comparatively empty lumen so that almost pure juice is obtained. The same variation in maximal acidity has been observed in normal persons after histamine (2). We know that the stomach tends to secrete a juice of high and relatively constant acid content it is this juice, comparatively undiluted, which we recover in these tests. On the other hand, in the oatmeal gruel test the stomach

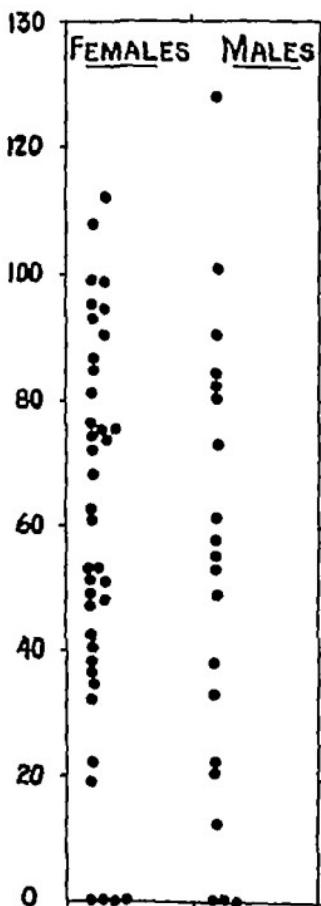


FIG 3 RESULTS OBTAINED BY FARR WITH LIEBIG'S EXTRACT TEST  
HIGHEST FREE ACID ATTAINED

secretes into a large volume of approximately neutral fluid containing buffer substances. Nevertheless the volume of oatmeal gruel and the degree of buffering do not seem to determine the maximal acidity attained after such a meal for the composition and volume of the meal

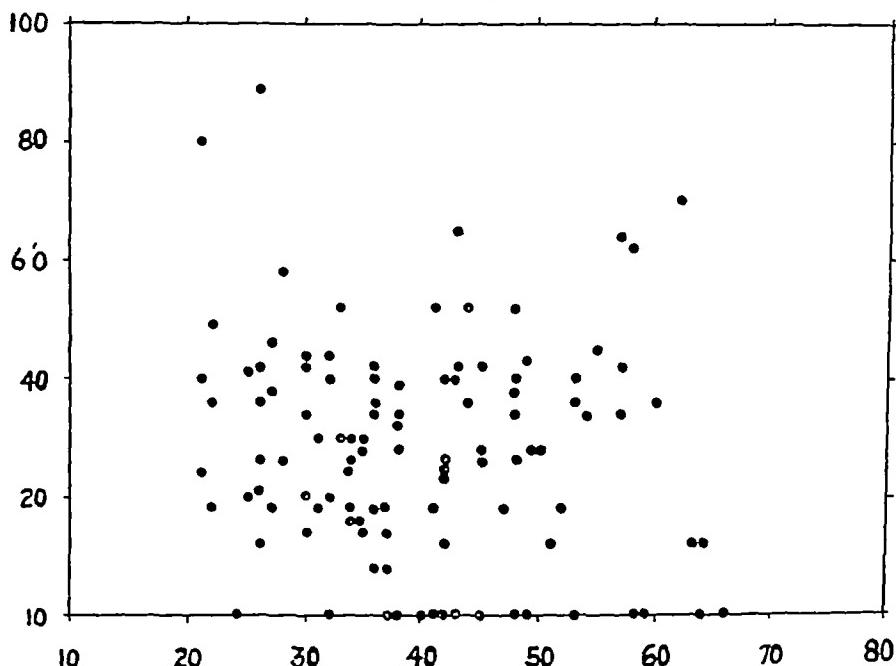


FIG 4 RESULTS OF GRUEL MEAL ON WHOLE GROUP EXCLUSIVE OF CASES WITH DUODENAL ULCER AND DUODENITIS

Age plotted as abscissae against highest free acid as ordinates

may be varied without materially affecting the degree of maximal acidity attained. The gastric response after oatmeal gruel gives evidence of the operation of the mechanism emphasized by Michaelis (4), "Under normal conditions the gastric secretion aims at reaching a definite pH, and the amount of secreted HCl necessary for this purpose differs according to the acid combining capacity of the food." The effectiveness of this mechanism is limited in the test with histamine and Liebig's extract, because the stomach lacks a buffer content to permit it to operate. Since this tendency to attain a definite pH is masked when the histamine test is used, quantitative measurement of maximal gastric acidity, after histamine, is of little significance or diagnostic value. This conclusion has no bearing on the demonstrated value of histamine as a qualitative test of the stomach's ability to secrete acid.

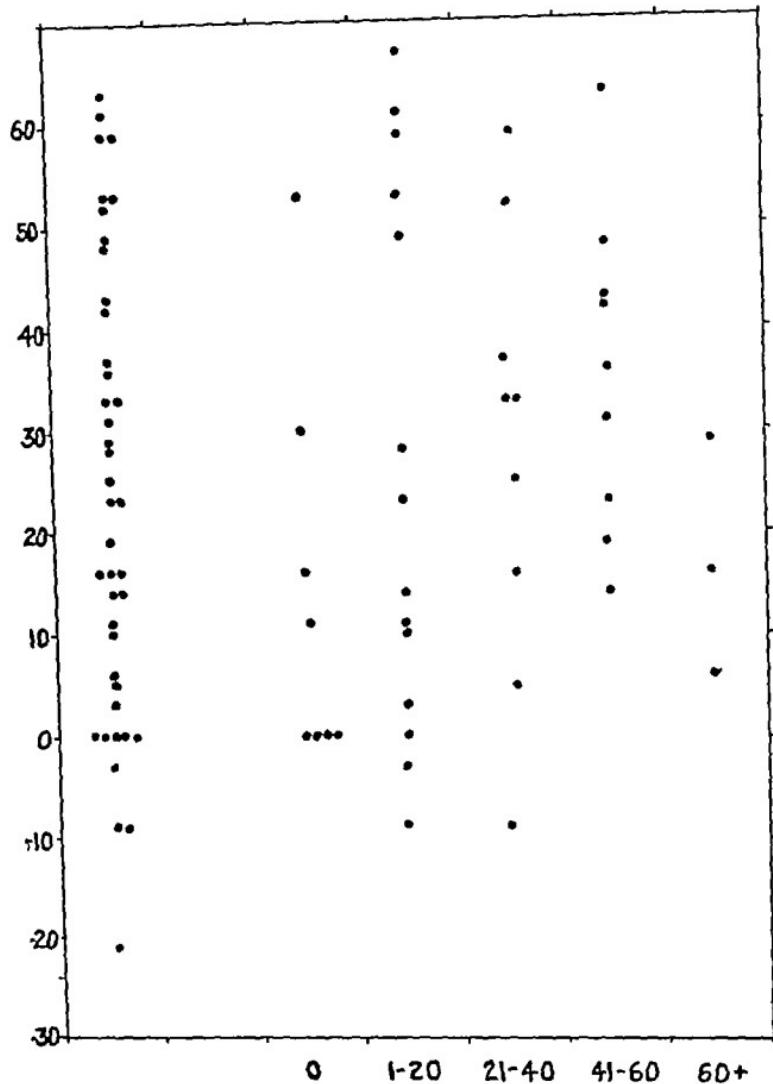


FIG 5 RESULTS OF HISTAMINE TEST AND GRUEL MEAL ON SAME PATIENTS

The column at the left represents the difference between the highest free acid after histamine and after gruel meal. In four cases the acidity was lower after histamine. In the columns to the right the difference between the maximal acidity in the two tests is plotted as ordinates against the degree of acidity after the gruel meal as abscissae. No correlation is apparent except in half the achylas.

*Sex and acidity*

In the histamine group, excluding the cases of duodenitis and ulcer, the males had an average maximal acidity of 45, the females, 42 (Figs 6 and 7) Bile stained samples were not used in the computations. This difference of the means of the sexes is less than its standard error and is without significance Kohiyar (6) found, in a large series of various gastro-intestinal diseases, that the acidities after a test meal

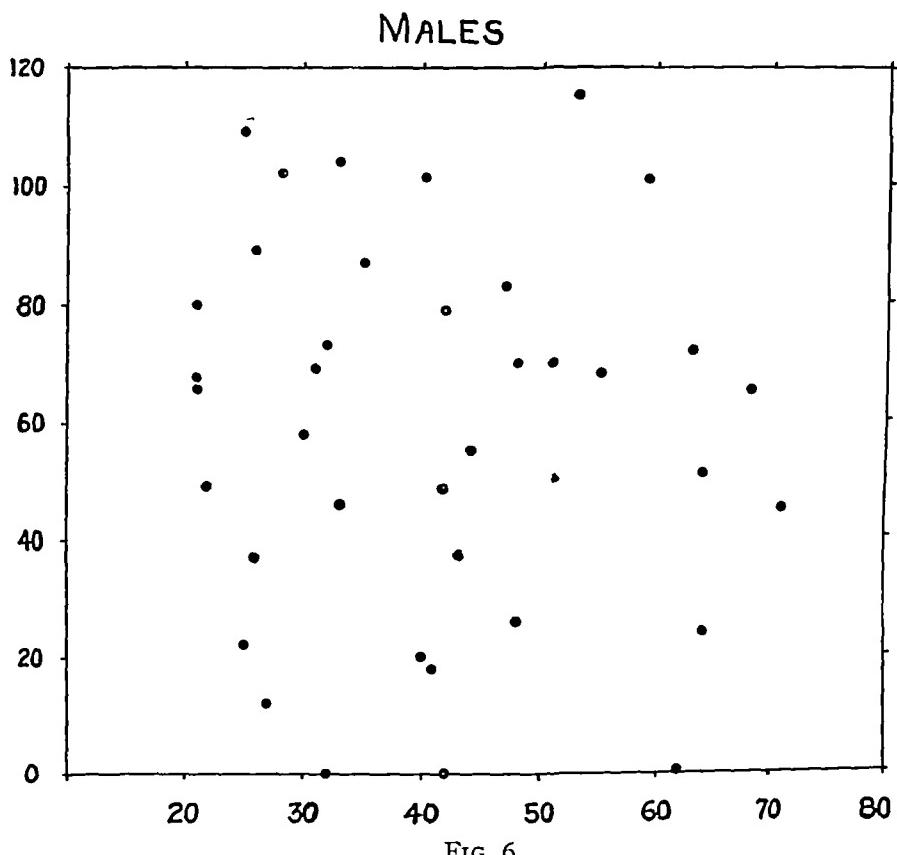
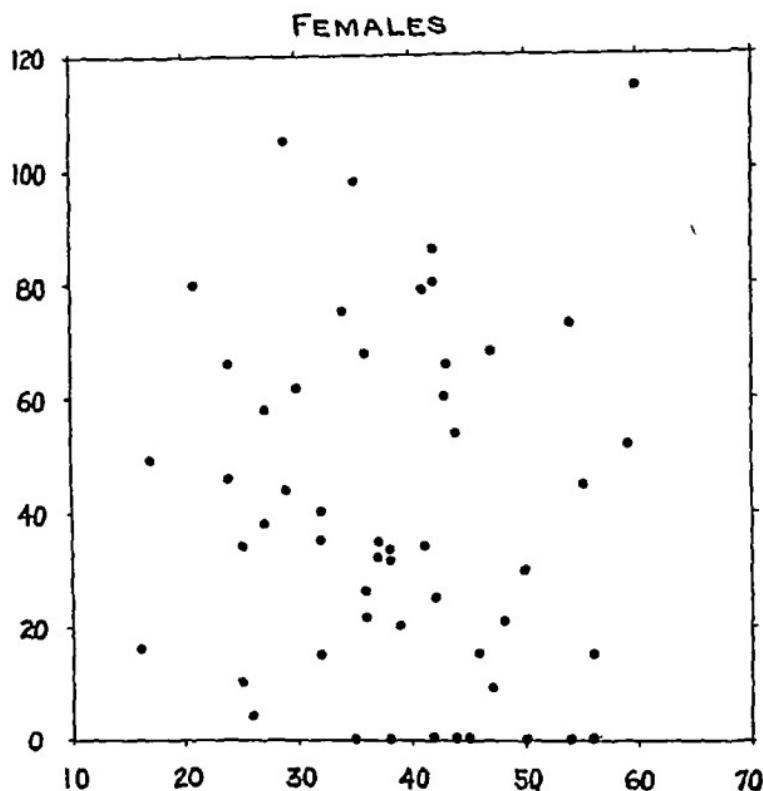


FIG 6

were higher in the male than in the female the males averaged 26, the females 20 Computation of his observations shows that the difference between his means was significantly greater than the standard error of the difference The sex difference was not significant in our series of gruel tests A sex difference, if it exists, may be related to the fact that males are more subject to diseases of the upper gastro-intestinal tract associated with high acidity



Figs 6 and 7 EFFECT OF SEX ON THE RESULTS OF HISTAMINE TEST,  
EXCLUDING CASES OF DUODENITIS AND ULCER MALES, FIG 6 FEMALES  
FIG 7

Age plotted as abscissae against highest free acid as ordinates

#### *Age and acidity*

An increasing incidence of achlorhydria with advancing age has been noted (7), but our data when analyzed statistically show no significant decline in acidity with increasing age either with the histamine or gruel tests

#### *Volume of secretion*

Numerous writers (8, 9 and 10) have called attention to variation in volume of secretion after histamine. For our whole group of cases, exclusive of those with duodenal ulcer and duodenitis, the highest 15 minute volume for each test is recorded in Table 3. The averages for

the various age decades fell between 30 and 40 cc per 15 minutes. This agrees well with the results obtained by continuous aspiration (11) (2). The duodenal ulcer and duodenitis group averaged about 54 cc per 15 minutes for each of the age decades from 20 to 50. There were so few cases of cancer in our group that no conclusions can be drawn from

TABLE 3

*Results of histamine test    Highest 15 minute volume of secretion and age of subject*

Highest volume per 15 minutes	Age					
	10-20	21-30	31-40	41-50	51-60	61-
cc. 91-100	cases	cases	cases	cases 1	cases	cases
81-90						
71-80						
61-70		2	2			
51-60	1	2	2	2	1	
41-50	1	5	5	5	1	
31-40		10	5	4	2	3
21-30		3	5	4	4	2
11-20		5	2	4	3	
5-10		1	1	3		
Average highest test	49	35	27	33	29	31

the tests that were made, although no striking differences were observed from the results of the whole group. The volumes ranged between 30 and 50 cc per 15 minutes.

No striking difference, which could be attributed to age or sex, was noted in the volume secretion for the whole group.

The relation between volume and acidity after histamine is recorded

in Table 4. There is no significant correlation. It may be noted, however, that the 10 cases with zero acidity had maximal secretion rates below 30 cc per 15 minutes, whereas 36 of the 107 cases with measurable acidity had maximal secretion rates above 30 cc per 15 minutes. Achlorhydrias, therefore, usually have a low volume of secretion.

TABLE 4  
*Results of histamine test Volume-acidity relation*

Acidity cc. N/10	Volume cc. per 15 minutes									
	1-5	6-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90
cases	cases	cases	cases	cases	cases	cases	cases	cases	cases	cases
131-140										
121-130										
111-120			1	3		1				
101-110	3	3	3	1	2					
91-100		1		1	1	1				
81-90		4	2		3	2				
71-80	1	1	6	1	1					
61-70		5	4	3	1		1			
51-60		2	3	1	1	1				
41-50	2	1	3	3	3	1	1		1	
31-40	2	4	2							
21-30	1	1	3	2	1	1				-
11-20		1	2	4						1
1-10					2					
0	2	2	5	1						

## SUMMARY

1 The maximal free gastric acidity after histamine and after Liebig's extract in a miscellaneous group of patients varied, to a far greater extent, from the mean value than it did after the gruel meal in a similar group

2 No correlation could be recognized between the maximal free acidity after histamine and after a gruel meal when the two tests were done on the same group of patients

3 The variations in the acidity after histamine could not be correlated, except in the achlorhydrias, with the diagnoses of the cases

4 No significant variations in acidity or volume secretion after histamine were noted in correspondence with the age or the sex of the patients

5 The volume of secretion after histamine was greatest on the average in the group of duodenal ulcer and duodenitis cases, but it bore no direct relationship to the degree of acidity, except that it was lower in those cases with zero acidity

## CONCLUSIONS

1 The histamine test, though valuable to determine the ability of the stomach to secrete acid, is not serviceable as a routine method of gastric analysis, because the variations in its results are great without obvious cause

2 The gruel meal has an advantage as a routine test of gastric acidity in that its results may be more nearly correlated with the clinical diagnoses

3 The explanation for the superiority of the gruel meal probably lies in the fact that it contains buffer substances which permit the stomach to adjust the acidity of its total contents to a more definite level, a level which is correlated with existing conditions

We wish to express our appreciation to Dr J Harold Austin for advice and criticism in this work

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## MINERAL EXCHANGES OF MAN

### III MINERAL METABOLISM DURING TREATMENT OF A CASE OF POLYCYTHEMIA VERA<sup>1</sup>

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The hemolytic properties of phenylhydrazine and phenylhydrazine hydrochloride were first demonstrated by Hoppe Seyler (16) in 1884. During the following thirty four years the drug or its derivatives were used to some extent in the production of experimental anemia in animals, but it was not until 1918 that it achieved a place in human therapy through publication of experiments by Eppinger and Kloss (11) who employed it to bring about destruction of erythrocytes in polycythemia vera. Within recent years a number of metabolic studies have been reported by investigators employing phenylhydrazine or its derivatives in the treatment of this disease. These studies have dealt mainly with changes in the physical and chemical properties of the blood, with studies of renal and liver function and of nitrogen metabolism, before, during and after treatment with hemolytic agents. So far as we have been able to ascertain there have been no reports on the mineral exchanges during a period of blood destruction resulting from the administration of phenylhydrazine derivatives to patients with erythremia.

For the past three years we have had a patient with polycythemia vera under observation.

On two occasions he has been admitted to the hospital for study and on one of these admissions the exchanges of nitrogen, phosphorus, calcium, magnesium and iron were followed during blood destruction.

<sup>1</sup> Expenses of this investigation were defrayed by a grant from the Fluid Research Fund of the Rockefeller Foundation.

MINERAL METABOLISM IN POLYCYTHEMIA VERA

The pertinent facts relative to his hospital admissions are summarized in the following case report

CASE REPORT

F D No 17059 A garage mechanic, 55 years old, was admitted July 28, 1928 complaining of severe pains in the lower back. The pain had been most acute for four days, lancinating in character, felt in both sides of the lumbar region radiating into the flanks. Motion was greatly limited and he was obliged to hold the back stiffly.

The past history and system review revealed that there had been a severe injury to the spine in an accident at the age of 11. He had had typhoid fever at the age of 6 years. At 18 years he had a severe gonorrheal urethritis, and at 25 years a febrile polyarthritis presumed to be acute rheumatic fever. The patient's wife stated that at various times during the six years from 1922 to 1928 he had shown an extremely high color, a beet-red or crimson appearance of face, hands and feet, particularly in cold weather. In March, 1928, there was a marked eruption on the face with intense itching followed by scaliness which lasted for two months. The family history was interesting in view of the fact that the patient's father apparently suffered from a similar condition (19).

ure acid 5.7 mgm., creatine 3.2 mgm., blood sugar 95 mgm. per 100 cc. The CO combining power was 57 volumes per cent. The blood calcium was 11.7 mgm. per 100 cc. The plasma contained no excess of bilirubin. The serum proteins were as follows: albumin 5.67 per cent, globulin 1.5 per cent, total 6.57 per cent.

The phenyl-sulphophthalimide excretion was 55 per cent in two hours. Two tests of the basal metabolic rate revealed it to be normal minus 2.5 and minus 4.5 per cent of average normal. The patient was given treatment by diathermy to the back with rapid improvement in comfort. The erythema was treated with phenylhydrazine hydrochloride, of which 2.4 grams were given in nine days. Blood destruction did not become apparent until 6 days after the drug was discontinued; when jaundice was noted the urine became highly colored, the blood counts and hemoglobin began to decrease, and leucocytosis increased. The blood count reached a minimum 27 days after the phenylhydrazine was discontinued, at which time the red cell count was 3.2 millions and thereafter it slowly rose again. At the height of the blood destruction the urine gave a positive reaction to guaiac. Subsequently the albuminuria disappeared, and bile pigments diminished and the urine became again normal.

The patient was readmitted on November 19, 1928, for a second course of treatment by blood destruction with a hydrazine derivative. At this time he was admitted to the metabolism unit for a study of the mineral metabolism during blood destruction.

#### METHOD

Blood chemistry. Hemoglobin was determined by Sahli's method (100 per cent hemoglobin taken as 15 grams hemoglobin per 100 cc. of whole blood); urea nitrogen by the method of Folin and Wu (12); sugar and ure acid by Benedict's methods (6); carbon dioxide combining power of the plasma by the method of Van Slyke and Neill (25); serum calcium by Clark and Celpi's (2) modification of the Tisdall method; serum proteins by the method of Wu and Long (26); ketone index by the method of Cutten, Emerson and Goodrich (9); total blood volume, cell volume, and plasma volume by the use of "brilliant malachite" as described by Hooper et al. (15).

*Nitrogen excretion.* The methods employed for the estimation of the excretions of nitrogen, phosphorus, calcium, magnesium, and iron were the same as reported in a previous publication (4) with the following exception: analyses of the diets given the patient were not made. These diets were, however, very similar in composition to sample diets previously analyzed in our laboratory. An empirical correction has therefore been introduced for the calculation of the nitrogen and calcium intake values. These values have been reduced approximately 6 per cent and 15 per cent respectively below the intake figures as obtained by calculation from tables pub-



administration of 2.9 grams of the drug compared favorably with those obtained during the previous period of treatment with phenylhydrazine hydrochloride (see case report). The total blood volume decreased from 7,790 cc. to 5,560 cc., the cell volume decreased from 5,330 cc. to 2,140 cc., a decrease of 3,190 cc. of cells and the plasma volume increased by 960 cc. or from 2,460 to 3,420 cc. Coincident with the changes in blood volume there was a decrease in hemoglobin from 141 per cent to 91 per cent and a fall in the erythrocyte count from 6.4 million to 3.8 million per cmm. The reductions in total blood volume,

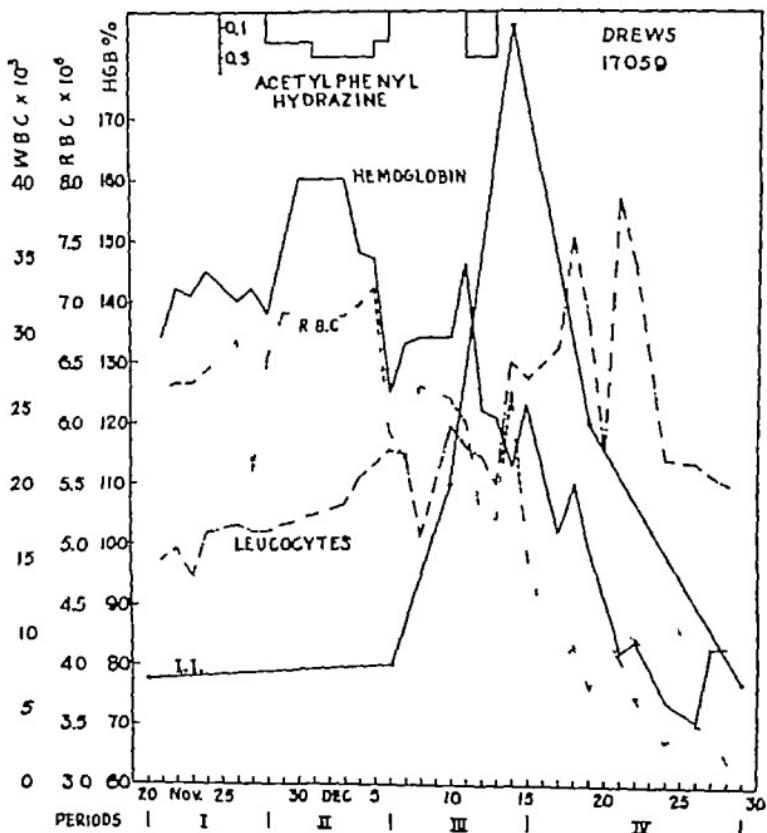


CHART 1 HEMOGLOBIN ERYTHROCYTES LEUCOCYTES AND ICTERUS INDEX (I.I.)

Units in scale on left refer to icterus index in units as well as to leucocytes in thousands

cell volume, hemoglobin and erythrocyte count are in accord with the studies of Brown and Giffin (7) and of Huffman (17).

Inspection of Chart 1 shows the rather pronounced delay in the onset of definite blood destruction following the administration of moderate daily doses of the drug. Long (18) has considered this point and suggested giving small doses, 0.1 gram daily until a total of 1 gram has been taken, and then waiting three days to see whether the effect has become cumulative. Giffin and Conner (13) have called attention to the fact that small daily doses of phenylhydrazine hydrochloride totaling not more than 3.5 grams are usually effective as an initial course of treatment. Experience with our patient is in accord with these opinions and also suggests that in certain individuals it may be desirable to wait as long as a week following a course of phenylhydrazine in order to ascertain whether the hemolytic agent will take effect.

Elevation of the serum bilirubin during the period of active blood destruction has been said to occur quite consistently and jaundice is not uncommon (Harrop (14)). Determinations of the icterus index on patient F.D. showed it to be markedly increased with the onset of blood destruction. Distinct jaundice was present at the time maximum values for icterus index were obtained and the urine was deeply pigmented throughout the period of bilirubinemia.

*Iron metabolism.* A consideration of the iron metabolism in connection with blood destruction in this disease is of interest. The remarkable ability of the body to conserve its iron supply is well known. Lichtwitz (quoted by Thannhauser (23)) has estimated that about 12.5 grams of hemoglobin are broken down daily in the body and converted into bile pigment. The amount of iron liberated by this destruction of hemoglobin corresponds to about 42 mgm of Fe, a quantity five or six times greater than the daily iron excretion of a man during starvation. Furthermore, it has been shown that even a proportionately much greater destruction of hemoglobin in dogs caused by the administration of hemolytic agents results in no appreciable loss of body iron (Samuely (21), Dubin and Pearce (10)). Reasoning from analogy one would be led to suppose that the treatment of human polycythemia with phenylhydrazine derivatives (which is essentially the same as the production of an experimental anemia in animals with hemolytic agents) would not result in any considerable increase in iron excretion.

This in fact appears to be the case. Patient F. D. remained essentially in iron balance during the first 16 days of the experiment with normal amounts of iron excreted in urine and feces. In the following 9 days there was a marked increase in the amount of iron excreted in

TABLE II  
*Iron metabolism*

Period date	Iron in food	Iron excreted	Iron balance	Medication	Remarks
November 20 to 27 I 8 days	mgm	mgm	mgm.		
		Urine 44 Feces 111.8			
	106.3	Total 116.2	-9.9	None	
November 28 to December 5 II 8 days		Urine 11.9 Feces 84.8		Acetylphenyl hydrazine	
	100.4	Total 96.7	+3.7	21 grams	
December 6 to 14 III 9 days		Urine 38.4 Feces 54.2		Acetylphenyl hydrazine	Reduction in circulating hemoglobin approximately 900 grams equals 3 grams Fe
	118.3	Total 92.6	+25.7	0.8 gram	
December 15 to 28 IV 14 days		Urine 25.3 Feces 66.2			
	178.9	Total 91.5	+87.4	None	
	39 days	503.9	397.0	+106.9	

the urine which is probably to be accounted for on the basis of hemoglobinuria occurring at the time of maximum blood destruction. Coinciding with the increased elimination of iron in the urine there was a decrease in the quantity of iron found in the feces which more than compensated for urinary loss. Whether or not this diminished fecal excretion was more than a matter of chance is not known. However, during the final period of 14 days the same general relationship between urine and fecal iron excretion was noted, although the total urinary iron for this period was less than for the preceding 9 days. During the final 23 days of the experiment which included the entire period of blood

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rived from red cell disintegration. If this is true and the reduction in quantity of erythrocytes as estimated by blood volume determinations is approximately correct, then a negative nitrogen balance of about 198 grams resulting from red cell destruction might have been expected.

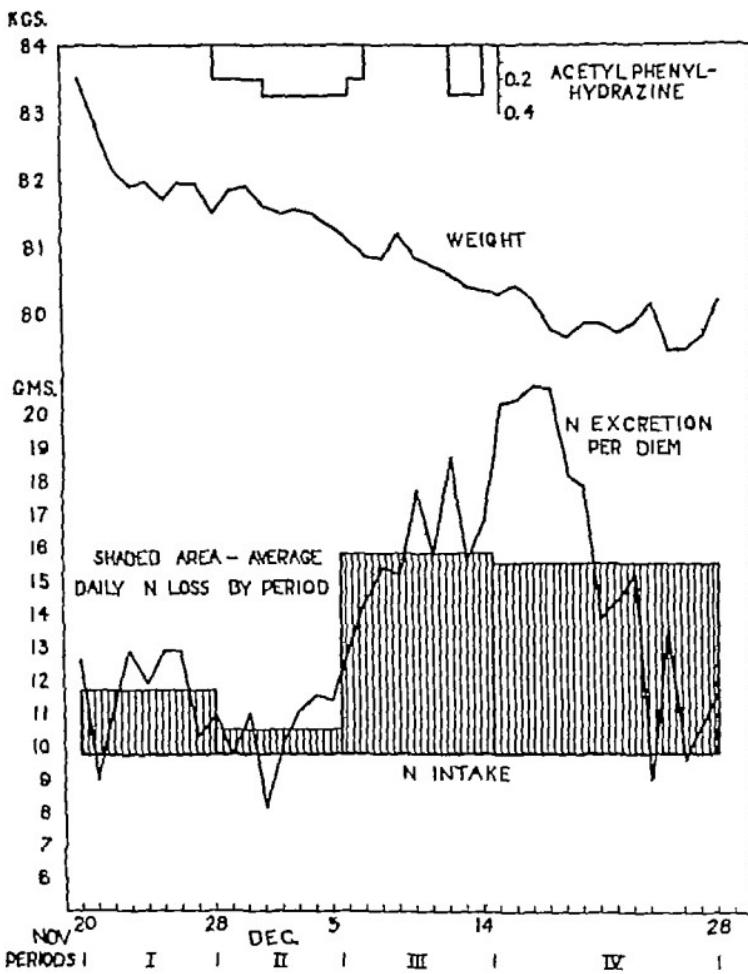


CHART 2 NITROGEN BALANCE

Equilibrium was practically established during the second period. Comparison with Chart 1 shows that the marked negative nitrogen balance coincides with the rapid decrease in hemoglobin and erythrocytes and with the maximum values for icterus index.

destruction, there was a positive iron balance of 113 mgm. From data furnished by hemoglobin and blood volume determinations it has been estimated that during these 23 days the circulating hemoglobin was decreased by approximately 900 grams (see Table I) and that 3 grams of iron must theoretically have been available for excretion or deposition in organs and tissues. The destruction of hemoglobin leading to this enormous increase in endogenous iron metabolism would actually seem to have provided a stimulus which caused retention of food iron as evidenced by the positive iron balance. The objection might now well be raised that since the iron balance was not followed during the subsequent period of blood regeneration one does not know that iron excretion was not delayed and that excretion of the iron liberated from hemoglobin did not eventually occur. Such a delayed iron excretion is not in accord with experimental evidence. Dubin and Pearce (10) followed the iron eliminated in the urine and feces of dogs kept on a constant diet, and noted no increase in the total iron excreted when anemia was produced by the administration of tolulylendiamine nor in the subsequent periods when the dogs were recovering from anemia. Moreover there is some evidence (2) to show that dogs kept anemic by daily administration of phenylhydrazine hydrochloride for a period of 5 months have increased deposits of iron in the liver and spleen when killed subsequent to recovery from the anemia. These facts are in accord with our iron balance data in the human subject.

#### *Nitrogen and phosphorus metabolism*

Huffman (17) has shown that patients with erythremia on a fixed diet showed negative nitrogen balances during the period of erythrocyte disintegration brought about by the administration of phenylhydrazine. The nitrogen balance in patient F. D. (Chart 2) was negative throughout the period of observation although his activity was minimal and he was receiving a protein intake of about 61 grams per diem with a daily caloric value for his diet of about 2400 calories (Basal requirement 1755 calories). The daily nitrogen excretion indicates that maximum nitrogen loss occurred during the periods when maximum values for icterus index were obtained or when blood destruction was most marked (Charts 1 and 2). It seems reasonable to assume that a large portion of the negative nitrogen balance was de-



The observed negative nitrogen balance (see Table III—Periods III and IV) was, however, only about 134 grams. It would seem then that some nitrogen containing fraction or fractions of the disintegrated erythrocytes, were retained perhaps to be reutilized in the formation of new hemoglobin.

Examination of the data pertaining to phosphorus metabolism (Table III) shows that during the first 16 days of the experiment the patient remained practically in phosphorus balance although there was a loss of both nitrogen and calcium. A distinctly negative phosphorus balance was noted only in Period III. Since destruction of blood occurred not only in this period but also during a considerable part of the subsequent one, a more complete picture of phosphorus metabolism at this time is obtained by considering the final 23 days of observation as a single period. With a loss of 134 grams of nitrogen there was retention of 1.31 gram of phosphorus. The small amount of calcium (1.68 gram) retained simultaneously could have accounted for a retention of 0.87 gram of phosphorus deposited as tertiary calcium phosphate in bone. The positive phosphorus balance at first appears surprising in view of the relationship between "theoretical" and actual phosphorus balances shown by Aub and his collaborators (3) (1). The assumption has already been made that most, if not all, of the negative nitrogen balance resulted from red blood cell disintegration, this being the case, the ratio of nitrogen to phosphorus in the excreta would be about 80:1 instead of 17:1 as might have been expected had the nitrogen excretion been dependent upon the catabolism of other body tissue (exclusive of bone). Such a ratio must necessarily materially decrease the excretion of phosphorus associated with nitrogen. The expected negative phosphorus balance therefore becomes so small as to be within the limit of error of the method used in calculating the balances. Finally, as in the case of nitrogen, it is possible that some of the phosphorus-containing fractions of the erythrocytes escaped excretion and remained to take part in the regenerative process during the subsequent period of recovery from acetylphenylhydrazine intoxication. Aside from speculation of this sort, it is evident that phosphorus was not lost from the body in appreciable amounts during the period of erythrocyte disintegration.

TABLE III  
*Nitrogen, phosphorus, calcium and magnesium metabolism*

Period date	Nitrogen			Phosphorus			Calcium			Magnesium			Medication
	In food	In ex creta	Balance	In food	In ex creta	Balance	In food	In ex creta	Balance	In food	In ex creta	Balance	
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	
November 20 to 27 I 8 days*	78.0	93.8	-15.8	8.71	8.02	+0.69	4.65	6.84	-2.19	1.90	2.44	-0.54	None
November 28 to December 5 II 8 days*	78.0	84.3	-6.3	8.52	8.53	-0.01	4.43	6.20	-1.77	1.95	2.49	-0.54	Acetylphenyl hydrazine 2.1 grams
December 6 to 14 III 9 days*	88.1	142.7	-54.6	9.92	10.98	-1.06	5.37	6.17	-0.80	2.16	3.08	-0.92	Acetylphenyl hydrazine 0.8 gram
December 15 to 28 IV 14 days*	137.0	217.0	-80.0	15.56	13.19	+2.37	7.88	5.40	+2.48	3.46	3.02	+0.44	None
*39 days*	381.1	537.8	-156.7	42.71	40.72	+1.99	22.33	24.61	-2.28	9.47	11.03	-1.56	

\* Totals for the four periods given at foot of each column

*Calcium and magnesium metabolism*

*Calcium* The average daily calcium intake for F D amounted to about 0.57 gram Daily variation from this average figure was small, so that although the individual balance periods were of different lengths, the calcium intake per period calculated on a per diem basis was almost identical Sherman (22) has shown that the minimum of actual need for a man of 70 kgm is about 0.45 gram Ca per diem For a man of approximately 80 kgm (the weight of F D ) this would be about 0.51 gram Ca a day, a quantity which very closely approaches the actual amount fed From this fact alone it might be expected that F D would show both positive and negative balances, which would tend to equalize each other if observations were continued for a sufficient length of time, assuming of course that the effect of such other factors as disease, medication, acid-base values of diet, activity, vitamins, etc , supposed to influence calcium metabolism could be excluded It will be noted (Table III) that negative calcium balances were obtained during the first three periods and that these negative balances became progressively less until in the final period calcium was retained The net result was a loss of 2.28 grams of calcium in 39 days Nothing abnormal was noted in the partition of excreted calcium between urine and stool The daily urinary calcium excretion was greatest (132 mgm ) in Period III which marks the onset of blood destruction, and least (73 mgm ) in Period IV at the time calcium was being retained Such variations are probably within normal limits and of no particular significance

Two determinations of serum calcium made prior to the onset of blood destruction gave values of 11.5 and 11.9 mgm per 100 cc which are within normal limits for the method used and are in agreement with the findings of Benedict and Turner (5)

*Magnesium* balances paralleled the calcium balances quite closely and it would appear that whatever factors were operative in causing a loss of calcium during the first three periods may also have resulted in a loss of magnesium The minimal magnesium requirement sufficient to maintain a man of average size in balance does not appear to be known It is readily conceivable however that it is subject to considerable individual variations, as is the case with other minerals, and may be influenced by many factors, not least of which may be the absolute level of intake in the diet

The average daily magnesium intake as found by Sherman (22) in a study of 150 American dietaries was 0.34 gram per man per day and per 3000 calories. Studies made by Carl Tigerstedt (24) in Finland indicate a much higher daily magnesium intake by men in that country (0.863 gram per day and per 2500 to 3500 calories). The subject of this investigation received a daily average of 0.243 gram of magnesium in his diet. Magnesium losses during the first two periods were almost identical 0.542 and 0.543 gram respectively. During the third period the loss amounted to 0.919 gram and in the final period 0.437 gram of magnesium was retained. The loss of magnesium during the entire period of observation was considerably greater in proportion to intake than that for calcium, amounting to 16.5 per cent of the total intake, while that for calcium amounted to 10.2 per cent of the total intake.

Considering the length of time the patient was under observation (39 days) and in view of the fact that retention of calcium and magnesium occurred during the final period it is probable that the net losses of 2.28 grams of calcium and 1.56 gram of magnesium during the four periods, were not associated with any marked disturbance of the factors governing the metabolism of these two elements.

It is rather interesting to note that positive balances for all the elements studied (except nitrogen) were obtained only in the final period. We are inclined to regard this phenomenon as an expression of anabolic activity on the part of the body tissues since the effect of acetylphenyl hydrazine had apparently ceased prior to the end of this period. It is realized however that one must exercise extreme caution in drawing any conclusions from such limited experience.

#### SUMMARY

1. The administration of 2.9 grams of acetylphenylhydrazine in doses of 0.2 to 0.3 gram per day to a patient with polycythemia vera resulted in marked reduction of total blood volume, cell volume, hemoglobin and erythrocyte count. An increase in the leucocyte count, icterus index and plasma volume occurred during blood destruction.

2. The large increase in endogenous iron metabolism accompanying erythrocyte disintegration did not result in loss of body iron. Hemoglobinuria is believed to have accounted for increased iron excretion in the urine during blood destruction.

3 Marked loss of nitrogen took place during the periods of blood destruction but the loss did not seem of sufficient magnitude to account for all the nitrogen contained in the destroyed erythrocytes

4 Significant changes in the metabolism of phosphorus, calcium and magnesium were not demonstrated

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## INSULIN ABSORPTION BY THE CONJUNCTIVAL MEMBRANES IN RABBITS

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The disadvantages of repeated subcutaneous insulin injections in the treatment of diabetes mellitus are well known. Numerous attempts have been made to find a more satisfactory route which have largely centered around absorption from mucous surfaces. Generally the results have been uncertain, uneconomical and at times impracticable, in spite of alterations in insulin and its use in combinations with other substances. There are many excellent reviews in the literature covering this subject, and one especially complete by Stammers (1).

There is no reference in the literature concerning absorption of insulin from the conjunctival membranes. Success by this route would obviate certain of the major objections to the hypodermic method. The animal experiments herewith reported have been carried out to determine the practicability as to rate, duration and constancy of absorption of insulin usage by the instillation of it in the conjunctival sac. Such instillation cannot be made in animals without waste, which should not occur in man, so it is therefore impossible to give accurate quantitative expression to the relative effectiveness of insulin by this route as compared to the subcutaneous injections. It is felt that more accurate data as to the economic possibilities of this method can best be determined by actual studies on human diabetics. We are now engaged in such studies. For our work in the clinic we have started with dry insulin, dissolving it in a menstrum which both lessens the high pH of commercial insulin and frees it from phenol, which we believe makes it absolutely safe for conjunctival instillation. In addition we have developed a dropping device which eliminates the danger of trauma to the eye and measures the dose with fair accuracy.

## METHOD OF STUDY

Rabbits which weighed as near two kilograms as possible were selected for this work. Blood was obtained from the ear veins and sugar determinations made by the micro method of Folin. The rabbits were fasted from 12 to 24 hours, and the fasting sugar level determined just before each insulin instillation. Groups of animals were given dosages of 2, 3, 4, 5, 6, 7 and 8 drops of insulin respectively, each drop containing approximately 5 units. The subsequent blood sugar determinations were made at intervals as nearly as possible each hour after the instillations until the fasting level had been essentially regained.

TABLE 1  
*Variations in blood sugar of untreated animals*

Number	Body weight	Time of fast	Initial	Blood sugar (mgm per 100 cc)	
				Time after initial (number of minutes in parentheses)	
3	2.40	hours 14	115	126(60), 102(120), 101(180), 103(240)	
14	3.91	0	122	117(60), 120(120), 111(180), 124(240)	
15	2.85	0	134	124(60), 129(120), 118(180), 126(240)	
16	2.95	0	114	120(60), 116(120), 114(180), 107(240)	
17	3.30	0	110	92(60), 114(120), 109(180), 115(240)	
22	3.90	24	109	106(60), 108(150), 112(300), 110(420), 111(450)	
23	3.35	24	97	103(60), 100(120), 100(240), 104(360), 105(400)	
24	2.24	24	114	107(60), 116(120), 110(240), 110(360), 113(420)	

## DISCUSSION

From Chart 1 it will be seen that the results of conjunctival administration of insulin causes a fairly uniform and consistent drop in the sugar level in rabbits' blood over a period of about 4 to 5 hours. It must be borne in mind that there was a variable loss of insulin by failure to get the full dose in the rabbit's eye at every administration. Occasionally an animal did not get the expected drop in blood sugar level, which may have been due to the loss of too much of the insulin, or that a given rabbit would not respond for other reasons. It has been noted repeatedly by other investigators with subcutaneous administration of insulin that occasionally certain animals would not show the expected drop. Various explanations have been offered for this fact, and it is possible that some of these latter factors have played a part in the ineffectiveness of insulin in the eye at times.

TABLE 2

Effect of administering insulin in the eye

Number	Body weight	Time of fast	Quantity of insulin	Feeding	Blood sugar (mgl. per 100 cc)	
						After administration (number of minutes in parentheses)
47	190	24	2	160	101(60)	121(90)
48	1940	24	2	120	103(60)	118(120)
49	1760	24	2	86	87(60)	93(120)
50	2100	24	2	120	103(60)	84(90)
51	2000	24	2	131	90(60)	80(90)
52	2200	24	2	119	76(60)	85(90)
53	2290	24	2	127	80(60)	82(120)
54	2400	24	2	128	120(60)	74(90)
55	1910	24	2	136	122(45)	127(90)
56	1940	24	2	104	100(45)	96(90)
2	2,570	12	2	147	99(60)	99(60)
1	242	12	3	137	139(65)	120(120)
4	239	36	4	117	99(50)	96(120)
7	395	30	4	91	55(30)	93(180)
8	284	30	4	106	57(95)	60(125)
9	226	24	4	113	69(105)	57(170)
11	—	208	24	4	74(195)	86(250)
12	200	24	4	117	87(167)	81(222)
13	247	24	4	137	74(88)	75(156)
33	191	—	—	110	59(70)	67(130)
					71(77)	91(123)

## INSULIN ABSORPTION BY CONJUNCTIVA

TABLE 2 (*continued*)

Number	Body weight kgm	Time of fast hours	Quantity of insulin drops*	Blood sugar (mgm per 100 cc)	
				Fasting	After administration (number of minutes in parentheses)
31	1.83	24	4	111	86(82)
32	1.91	24	4	95	77(83)
34	1.83	24	4	114	81(45)
35	2.05	24	4	112	86(34)
36	1.57	24	4	98	77(36)
18	1.84	24	5	124	82(60)
5	3.35	24	5	103	56(153)
6	2.96	24	6	110	91(152)
10	2.50	24	6	127	64(107)
19	1.90	24	6	119	80(62)
37	1.94	24	6	127	78(45)
38	1.90	24	6	119	80(48)
39	1.89	24	6	121	70(50)
20	1.92	24	7	106	81(62)
21	1.91	24	8	111	59(61)
40	1.90	24	8	131	66(50)
41	1.93	24	8	119	71(50)
42	2.00	30	8	115	78(20)
43	1.700	30	8	99	63(21)
44	1.90	30	8	126	72(60)
45	2.07	30	8	100	66(55)
46	1.95	30	8	114	68(60)

\* In all this work a drop is roughly equivalent to 5 units of commercial insulin—340 units to 1 cc Prepared and furnished us by the Eli Lilly Co., Indianapolis

TABLE 3

Effect of intravenous administration of insulin.\*

Number	Body weight	Time of fast	Quantity of insulin	Blood sugar (m.m., per 100 cc.)	
				Fasting	After administration (number of minutes in parentheses)
25	1.85	hours	1/24	110(62)	86(265)
	0		3	70(61)	122(266)
26	1.70	0	3	44(40)	54(102)
				50(67)	87(249)
28	1.85	16	3	121	61(111)
	2.5	16	3	101	88(277)
30					107(377)

TABLE 4  
Effect of subcutaneous administration of insulin.\*

Number	Body weight	Time of fast	Quantity of insulin	Blood sugar (m.m., per 100 cc.)	
				Fasting	After administration (number of minutes in parentheses)
27	2.22	hours	1/24	110(46)	65(108)
	1.6		3	87(38)	62(92)
29	1.84	16	3	111	79(253)
					64(247)

\* Tables 3 and 4 are merely inserted to give a comparative idea of the action of insulin by eye as against intravenous and subcutaneous injections.

There can be no doubt, however, that the response was not always uniform. A few animals showed as much drop on a relatively small dose as others showed on a larger one. Occasionally a few of our animals showed symptoms of insulin shock.

The preparation of insulin which we used in these experiments was supplied to us by the Eli Lilly Co., Indianapolis. It contained 340

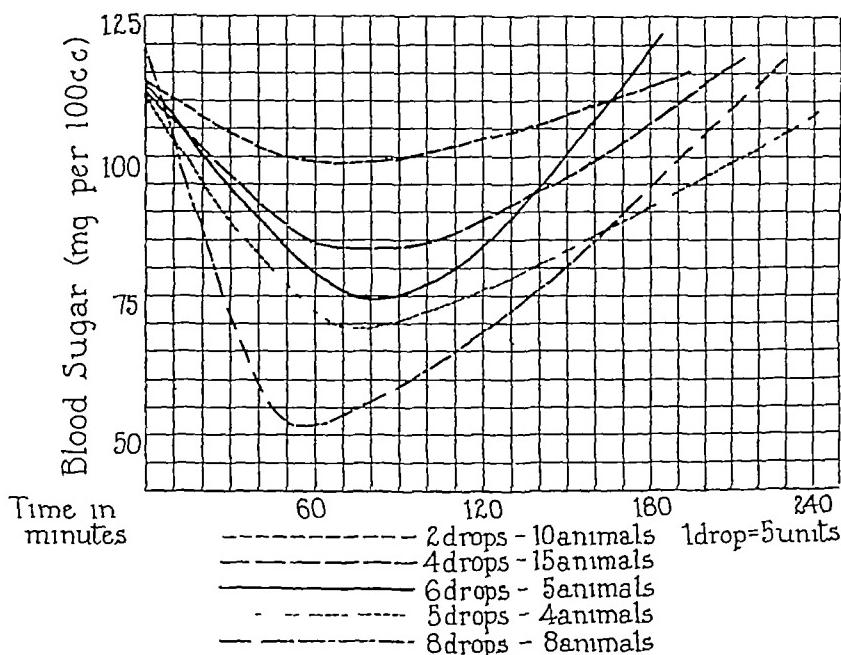


CHART 1 EFFECT OF CONJUNCTIVAL ADMINISTRATION OF INSULIN ON BLOOD SUGAR OF RABBITS

units to the cc. It had a pH of about 2.5, and contained 2 per cent of phenol. We noted at times a white scum form over the rabbit's eye immediately after the insulin was instilled. This we believe was due to the buffer substances in the lachrymal secretion neutralizing the instilled insulin. Insulin is insoluble in a neutral medium.

We have not sufficient data to venture a guess as to the effectiveness of insulin by eye in the rabbit as compared to subcutaneous injections. It is our impression, however, that it requires about 60 per cent more

We have seen no evidence to indicate that even commercial insulin is irritating to the eyes of rabbits.

## CONCLUSIONS

Insulin instilled into the conjunctiva is absorbed with great rapidity and gives rise to a fairly constant and consistent fall of blood sugar levels in rabbits.

The drop in blood sugar level persists for from 4 to 5 hours as against from 5 to 6 hours by the subcutaneous route.

Apparently it requires about 60 per cent more insulin by this method of administration than the subcutaneous route, although our experiments are not conclusive on this point.

We have seen nothing to make us believe that commercial insulin is irritating to the eyes of animals.

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## ELECTROCARDIOGRAPHIC STUDIES DURING ATTACKS OF ANGINA PECTORIS AND OF OTHER PAROXYSMAL PAIN

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(Received for publication July 13 1931)

The mechanism of the pain in angina pectoris has been the subject of speculation and investigation since the original report of this clinical condition by Heberden (9). Among the many theories there are two which today receive most support (1) Coronary origin—(a) myocardial ischemia due either to sclerosis of the coronary arteries and consequent loss of elasticity or to coronary artery spasm, (b) distension of the sclerosed coronary arteries with stimulation of the periarterial sympathetic nerve fibers and (2) Aortic origin—due to distension of the diseased supra sigmoid portion of the aorta with tension on the nerve endings in the adventitia (Wenckebach, Allbutt). Recent electrocardiographic observations made during transient attacks of angina have revealed inversion of the T wave or S-T segment of the curve, usually in leads I and II. There is immediate return of the curve to normal after subsidence of the pain. These electrocardiographic studies have been reported by several observers Clerc (3), Arrilaga (1), Bousfield (2), Feil and Siegel (4), Levy (5), Parkinson and Bedford (6) and Wood and Wolferth (7). The clinical observations have been substantiated by the experimental work of Wood and Wolferth (7) who produced "temporary and rapidly reversible electrocardiographic changes analogous to those seen in angina pectoris tracings." These changes were greater inversion of previously negative T waves in some experiments and the production of a high take-off in the S-T complex in others. These investigators obtained abnormalities in the T wave more readily in abnormal hearts. Clamping of the circumflex and posterior descending coronary arteries caused greater changes than obstruction of the ramus descendens or branches of the right coronary artery. Feil, Katz, Moore, and Scott (8) in a recent experimental

study obtained similar curves from ligature of the ramus descendens of the left coronary artery. These changes were most pronounced after further ischemia of the heart muscle was produced by obstruction of the inferior vena cava. These clinical and experimental data are strong evidence that myocardial ischemia is responsible for the changes in the T wave and S-T segment of the curves. Likewise, they indicate that the pain is probably due to or associated with myocardial ischemia.

In this report are given the electrocardiographic findings in eleven additional cases with transient anginal attacks. Clinically, these cases may be divided into four groups: (1) Patients with coronary sclerosis and who were not known to have had coronary artery thrombosis, (2) Patients who had in addition, an attack of coronary thrombosis previously, (3) Patients whose angina was associated with aortic regurgitation, and (4) Patients who had angina and who subsequently developed coronary artery occlusion.

The results of these observations during anginal attacks are summarized in Table I.

TABLE I  
*Electrocardiographic changes*

Case	Diagnosis†	T <sub>I</sub>	T <sub>II</sub>	T <sub>III</sub>	Depression of S-T Segment
1	C S				Leads 2 and 3
2	C S R C T				Leads 2 and 3
3	C S, O C T				Leads 1 and 2
4	C S, O C T				Lead 1
5	C S, O C T				Leads 1 and 2
6	C S	neg	neg		
7	A I		neg		
8	C S		neg		Leads 1 and 2
9	C S, O C T				
10	C S				
11	C S				

† C S—Coronary sclerosis R C T—Recent coronary thrombosis O C T—Old coronary thrombosis A I—Aortic insufficiency, rheumatic

#### CASE REPORTS

*Case 1* L F., female, aged 56. Diagnosis Coronary sclerosis with gradual obliterative arteritis and myocardial fibrosis. She had attacks of substernal pain with radiation to the left shoulder and down the left arm. These attacks had been recurring for two years, usually after emotional up-

set and were not precipitated by exercise. The blood pressure was usually 150/90 but during the attack rose to 170/100. The heart rate usually 80 rose to 120 and the cardiac mechanism remained normal. Physical examination revealed a heart normal in size and heart sounds that were feeble. The first heart sound at the apex was especially faint and gallop rhythm was present much of the time. During the latter part of the patient's illness there were attacks of pulmonary edema and the terminal illness was dominated by congestive failure. Postmortem examination revealed advanced arteriosclerosis of both coronary arteries with almost complete obliteration of the lumen of the ramus descendens. There was advanced sclerosis of the muscle of the anterior and lateral part of the left ventricle. An electrocardiogram taken on November 13, 1928 (Fig. 1 a) during an attack revealed normal mechanism, rate 136, the S-T segment in lead I rose after the completion of R and T was elevated 2 mm. The S-T segment in lead II was somewhat depressed and T was upright. Lead III shows depression of the S-T portion of the curve. An electrocardiogram taken after the attack subsided (Fig. 1 b) showed T 1 to be almost isoelectric. S-T 2 was slightly depressed. T 2 and T 3 were both upright. The rate was 100.

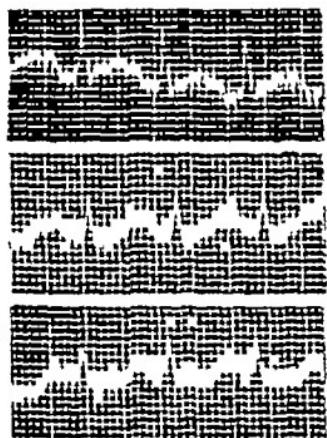


FIG. 1 a

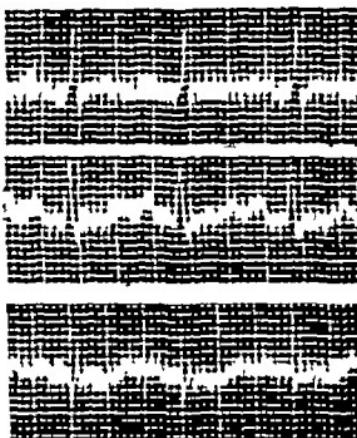


FIG. 1 b

*Case 8* C.H., male aged 62. Diagnosis Coronary sclerosis with angina pectoris recent coronary thrombosis. For several months he had attacks of substernal pain on effort, relieved by nitrites. A severe attack of substernal pain occurred three weeks before the electrocardiographic study was made. This attack was probably due to coronary thrombosis. The patient had had elevation of the systolic and diastolic blood pressure for approxim-

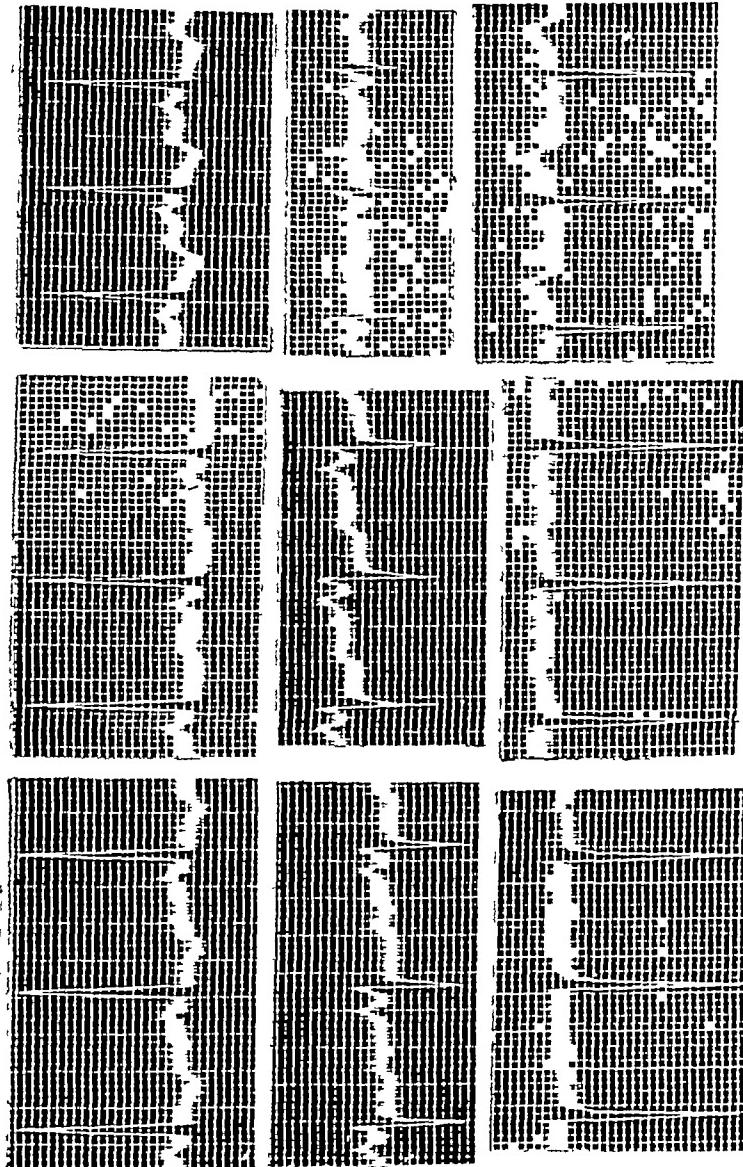


FIG 2, a

FIG 2, b

FIG 2, c

ately eighteen years (180/110) An electrocardiogram (Fig. 2, a) taken on December 29, 1929 revealed inversion of T 1 depression of S-T 2 and T 2 was upright T 3 was upright Immediately after this record was taken the patient complained of severe pain (Fig. 2 b) which showed a greater depression of S-T 2 There was less depression of the S-T segment in lead III The patient inhaled amyl nitrite and was relieved of pain Figure 2 c, shows a striking change T 1 is more deeply depressed S-T 2 is isolectric S-T 3 is slightly elevated and T 3 is larger

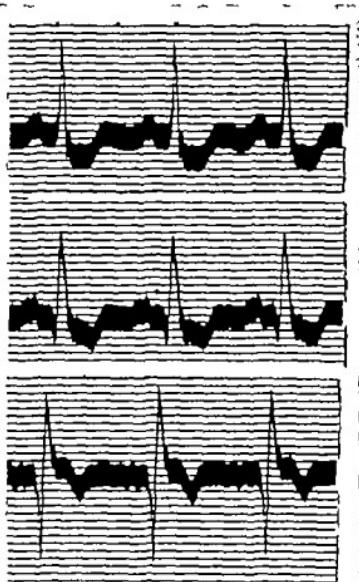


FIG. 3 a

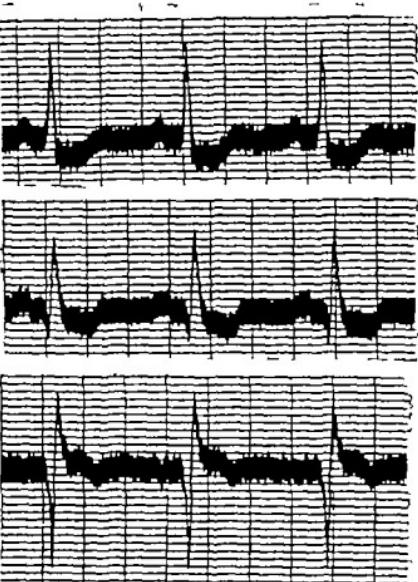


FIG. 3 b

**Case 3** J W male aged 61 Diagnosis Coronary sclerosis with angina pectoris old coronary thrombosis. Chief complaint substernal pain on effort with radiation to neck left shoulder and arm Symptoms dated from a sudden and severe attack of substernal pain August 1927 (1 year before observation) Generalized arteriosclerosis Blood pressure 150/100 Moderate enlargement of left ventricle An electrocardiogram taken on May 31 1928 during an attack of angina (Fig. 3 a) revealed a depression of the S-T segment in leads I and II A very deep Q is seen in lead III and S-T 3 has a high take-off and is curved with its convexity upwards A record taken a few minutes later after subsidence of the pain (Fig. 3 b) shows less depression of S-T in leads I and II

*Case 4* H M, male, aged 48 Diagnosis Coronary sclerosis, angina pectoris, recent coronary thrombosis Coronary thrombosis July 1929 Attacks of typical angina with effort and emotion Moderate cardiac enlargement Blood pressure 160/120 An electrocardiogram was taken one month after occlusion during an attack of angina Figure 4, a, shows considerable depression of the S-T segment in lead I, slight inversion of T 2 and a moderately high take-off of S-T 3 with inversion of T 3 Immediately after subsidence of the pain the electrocardiogram (Fig. 4, b) shows less inversion of S-T 1, greater inversion of T 2 and an altered QRS complex in lead III

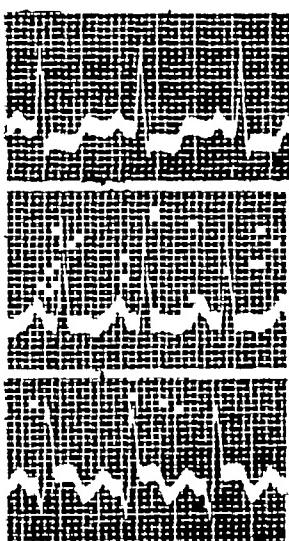


FIG 4, a

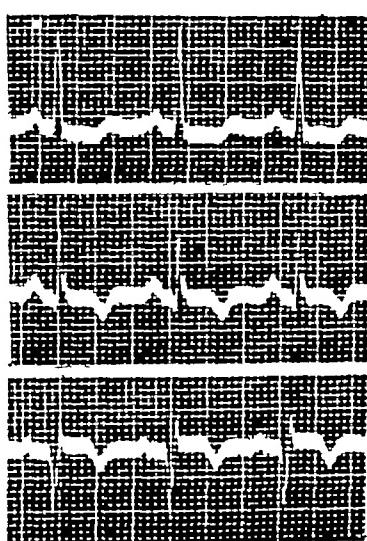


FIG 4, b

*Case 5* M F, male, aged 58 Diagnosis Coronary sclerosis, angina pectoris, old coronary thrombosis Had attack of coronary thrombosis April 28, 1927 Moderate cardiac enlargement to the left Blood pressure 200/110 Moderate generalized arteriosclerosis An electrocardiogram taken on August 30, 1930 (Fig. 5, a) during a severe anginal attack revealed a deeply depressed S-T segment in leads I and II and in lead III T is isoelectric A record taken after relief by inhalation of amyl nitrite shows very slight depression of S-T in leads I and II and in lead III T becomes depressed

*Case 6* M B, female, aged 53 Diagnosis Coronary sclerosis, angina pectoris Typical angina of effort in substernal region with radiation to the left shoulder and arm Duration six years Heart not enlarged Blood pressure 180/100 An electrocardiogram was taken on October 16, 1930

during an attack of severe angina. Figure 5 a shows the striking inversion of T 1 and T 2. A record taken immediately after relief by the inhalation of amyl nitrite shows the depression of T 1 and 2 (Fig. 5 b).

(Case 7) E. F., male aged 27, has had a history of free cardiac insufficiency (rheumatic) since 1932. He has had two attacks of angina pectoris. Chorea at the age of 19 (heart attack) and then again in 1940. He has had frequent attacks of rheumatic fever during succeeding years. For 7 or 8 years patient had had nocturnal attacks of pain in the subscapular region radiating to the left shoulder and down the

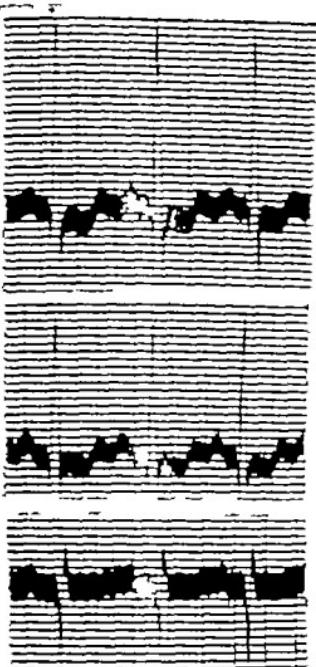


FIG. 5 a

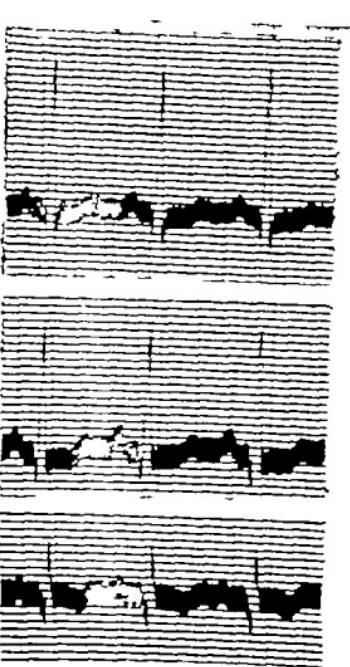


FIG. 5 b

left arm. There have been no signs of congestive failure. The blood Wassermann was negative. An electrocardiogram taken during an attack (Fig. 5 a) shows a slight inversion of the S-T segment in lead I and a deep depression of this portion of the curve in leads II and III; the S-T portion curving off below the iso-electric level. A record taken after the inhalation of amyl nitrite (which relieved from symptoms) shows a deeper inversion of T 1 and a less deep inversion of T 2 and 3. In addition the S-T portion of the curve started at a higher level in lead II and near the iso-electric level in lead III.

*Case 8* A C, female, aged 49 Diagnosis Coronary sclerosis with angina pectoris Pain in lower xiphoid region for two years with radiation to left shoulder and arm, related to effort and emotion Blood pressure 142/82 Wassermann negative An electrocardiogram taken as a control (Fig 8, b) shows left axis deviation and T 1 and 2 are upright T 3 is iso electric 1 cc of 1-1000 solution of adrenalin hydrochloride was injected subcutaneously This was followed by a typical attack of pain and a record taken 18 minutes after the injection (Fig 8, a) shows a depression of the S-T segment in leads I and II with a low "take off" In lead III the S-T segment is curved with the convexity directed upwards and T is inverted

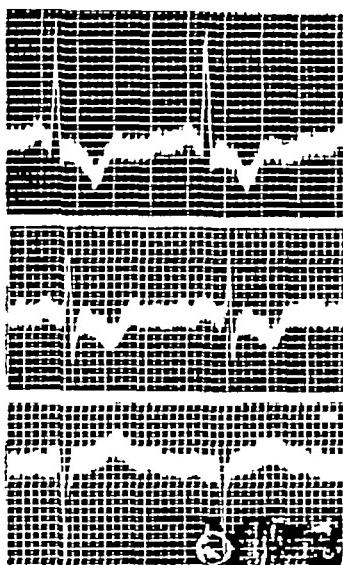


FIG 6, a

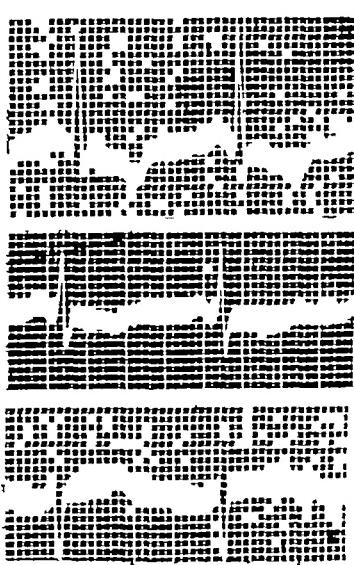


FIG 6, b

Three patients with coronary sclerosis and angina of effort showed no significant electrocardiographic changes

*Case 9* F R, female, aged 60, with typical substernal pain on effort Coronary thrombosis one year previously Blood pressure 156/84 Wassermann negative Electrocardiograms during pain showed no change in the T wave

*Case 10* M K, male, aged 58 Attacks of substernal pain of three years' duration caused by exertion Blood pressure 164/90 Patient later developed coronary thrombosis and died Electrocardiograms during attacks of angina showed no change

*Case 11* M K, male, aged 43 Diagnosis Coronary sclerosis with angina of effort Sense of substernal oppression related to exertion and emo-

tion Radiation of pain to both arms Duration one year Blood Wassermann negative Electrocardiogram taken after subcutaneous injection of 1 cc of adrenalin hydrochloride 1-1000 solution showed very slight changes in the T wave The patient suffered pectoral constriction

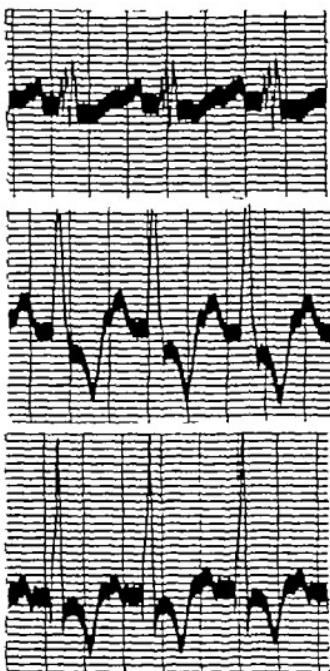


FIG 7 a

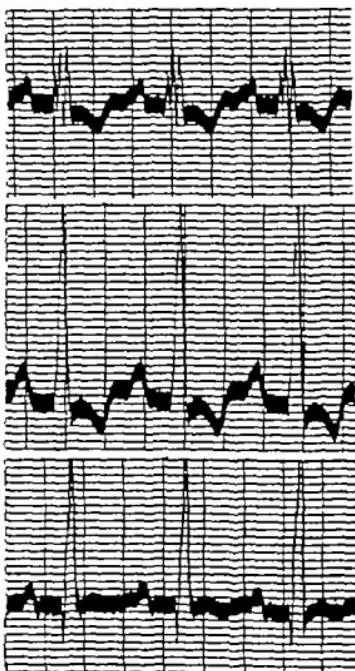


FIG 7, b

*Electrocardiographic observations made during paroxysmal pain  
not due to angina pectoris*

1 Labor pains Electrocardiograms were taken during active labor in five patients In each case tracings of the standard leads were recorded during an interval when the uterus was relaxed This was repeated while the patients were having severe uterine contractions which were visible and palpable Records were again taken after the uterine contractions had subsided In two cases records were obtained which showed some deviation from the normal which was present during both active contraction and during relaxation In these two cases records were obtained a few days postpartum for comparison

In none of the five cases was any inversion of the T wave or depression of the S-T segment of the curve observed during uterine contraction. In one case the T wave in lead III was depressed both during uterine contraction and relaxation. A record taken six days post-partum showed less inversion of T 3.

**2 Renal and biliary colic** One patient had severe biliary colic and was found at operation to have pericholecystitis with numerous adhesions. Records taken during biliary colic showed no deviation from the record taken after relief from pain.

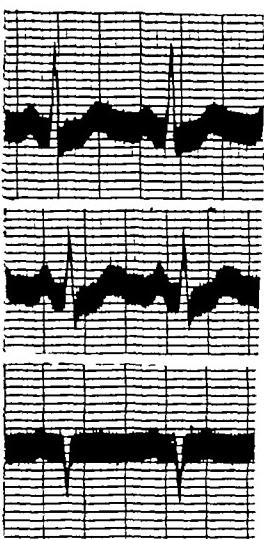


FIG 8, a

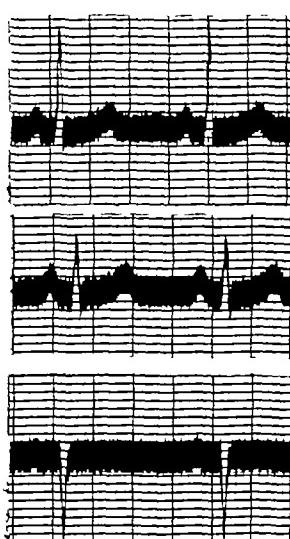


FIG 8, b

Two cases of renal colic were investigated. One who had severe colic for several days had marked sinus arrhythmia which disappeared with relief from pain. However, neither of these two cases presented any alteration of the S-T segment or T wave during pain.

#### DISCUSSION

The changes in the T wave and S-T segment which occur frequently (in more than 50 per cent of cases) during attacks of angina pectoris are indicative of myocardial changes which are not related to exercise (Wolferth, personal observations) or to reflex effects from pain as such (labor pain, gallbladder and renal colic). In one patient, with a

gastric ulcer and paroxysmal epigastric pain slight depression of the T wave occurred which varied in successive beats and was perhaps related to changes in the axis of the heart In eight cases electrocardiographic changes were associated with characteristic pain (due to effort or emotion) and subsided with rest and inhalation of amyl nitrite Myocardial changes may then be said to be associated with transient anginal attacks and this may be due to spasm of the coronary arteries or to transient variations in the coronary flow (reversal of coronary flow) The inversion of T or the S-T segment in the electrocardiogram during brief paroxysms of pain of angina pectoris gives additional evidence of the nature of the anginal attack and may be helpful diagnostically

#### CONCLUSIONS

Electrocardiographic observations are reported in eleven additional cases made during transient attacks of angina pectoris In eight of these patients, there was inversion of the T wave and (or) inversion of the S-T segment of the curve In some instances a negative T became more deeply depressed These changes disappeared after subsidence of the pain This evidence supports the conception that transient anginal attacks are due to myocardial ischemia, presumably due to alteration in coronary artery flow Electrocardiograms taken during other types of paroxysmal pain (labor pains, renal and gallstone colic) did not show any changes in the T wave or S-T portion of the curves

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## STUDIES IN CONGESTIVE HEART FAILURE

### XIII THE RELATION OF DYSPNEA OF EXERTION TO THE OXYGEN SATURATION AND ACID BASE CONDITION OF THE BLOOD<sup>1 2</sup>

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In a previous study (Harrison Turley, Jones and Calhoun (1931)) it has been shown that the degree of dyspnea produced by muscular effort is directly proportional to the ventilation and inversely proportional to the vital capacity. For a constant amount of exertion this ratio  $\frac{\text{Ventilation}}{\text{Vital Capacity}}$  was higher for patients with cardiac disease than for normal subjects. These results involve two problems

(1) Why does the individual with cardiac disease usually ventilate more than the normal does for the same exertion?

(2) How does decreased vital capacity tend to produce dyspnea?

The most generally accepted theory of respiratory control is that the increase in ventilation must be due to either an increased hydrogen ion concentration or increased CO<sub>2</sub> tension of the blood or respiratory center or of both. This in turn is supposed to be caused by insufficient aeration of the tissues or respiratory center because of decrease in cardiac output. In support of this are the conclusions of Sir James MacKenzie (1925) Means (1924) and Meakins and Davies (1925), that cardiac dyspnea is due to decreased blood flow through the brain.

If these views are correct it should be possible to find changes in oxygen saturation and acid base condition in the blood, especially in the blood flowing through the brain. The present paper presents the

<sup>1</sup> Aided by a grant from the National Research Council

<sup>2</sup> Presented in abstract at the Meetings of the Federation of American Societies of Experimental Biology April 9 1931 at Montreal Canada

results of a study of these variables made with modern methods upon a series of patients with cardiac dyspnea

#### EXPERIMENTAL

*Plan* The study included analysis of blood for O<sub>2</sub> capacity and content, of blood serum for pH and total CO<sub>2</sub> content, the determination of the ventilation of the subject, before, during and after a fixed amount of exertion, and the determination of the vital capacity. The source of blood varied as the work progressed. The first series was done on venous blood from the arm, the second series on arterial blood from the brachial artery and the third series on venous blood from the internal jugular vein. The details of blood sampling and the results for each series are grouped in accordance with the source, i.e., arm vein, brachial artery and internal jugular vein.

*Subjects* The subjects studied included seven normal males between the ages of twenty-five and forty, and twelve patients with cardiac disease of various types (usually hypertensive or syphilitic) in various stages. Some of the patients had only slight symptoms, others had had repeated breaks in compensation. The patients were classified according to the degree of cardiac disease, “+” refers to those individuals whose only symptom was dyspnea on exertion, “++” and “+++” designate respectively subjects with slight and moderate degrees of congestive failure.

*Blood Analyses* Approximately twenty to twenty-five cubic centimeters of blood were taken (the syringe containing mineral oil) for each sample. Part of this—six to eight cubic centimeters—was expelled into a small bottle containing sodium oxalate and mineral oil. This was used for determination of oxygen content and capacity by the Van Slyke-Neill technique (1924). The remainder of the blood was placed in a centrifuge tube under oil, the oil was replaced by melted paraffin, and the tube centrifuged. After centrifuging 20 to 30 minutes, mineral oil was run over the paraffin plug, the plug removed and the serum removed with a pipette containing oil to a Pyrex tube containing oil. The hydrogen ion concentration of the serum was determined by Cullen's method (1922) using the refinements reported by Earle and Cullen (1929). All readings were made at 20° C. in a constant temperature room with constant (“Daylight”) source of light and were corrected to

38° by subtracting 0.23 pH. All readings were made independently by two observers. In addition to the values read against the phosphate color standards the individual tubes of each series were compared against each other so that with these checks a difference of 0.02 pH may certainly be considered as true difference. The carbon dioxide content of the serum was determined by the manometric extraction apparatus of Van Slyke and Neill (1924).

Carbon dioxide tensions were calculated from the Hasselbalch formula (1912), using Van Slyke and Sendroy's (1927) absorption coefficients and a pK' value of 6.10.

*Ventilation.* These findings were obtained with a Tissot spirometer which was connected to a fairly comfortable face mask with the regular arrangement of valves. The vital capacity was measured on a Benedict-Roth spirometer.

*A The findings in the venous blood obtained from the arm  
(Tables 1 and 2)*

*Procedure.* The observations were made in the resting post absorptive state. The subject came to the laboratory without breakfast and rested in a comfortable chair for ten or more minutes. A face mask was applied and the expired air was collected for a five minute period. Venipuncture at the elbow was then performed, after injection of novacaine into the subcutaneous tissue, with a needle which had a well fitting stilette. (For this purpose a sixteen gauge lumbar puncture needle was cut off to a length of about 4 cm and re sharpened.) Stasis was usually necessary for the puncture. After the needle was well in the vein the tourniquet was removed, the stilette inserted and the needle fixed in place with small strips of adhesive plaster. Several minutes later blood was drawn into a syringe containing mineral oil, no stasis whatever being used. The subject then stood up and walked up and across and down from a platform two steps high and 64 cm broad at a rate of one "round trip" in twenty seconds for two minutes [More complete details of the exercise are given in the previous paper by Harrison Turley, Jones and Calhoun (1931). The exercise referred to in the present paper as "mild" is the exercise I of their paper, whereas "moderate" exercise is the exercise III of their paper.] He then sat down and a second blood sample was taken immediately.

A record was kept of the time at which the blood first appeared in the syringe and of that at which the sample was completed. It was usually possible to obtain the necessary twenty cubic centimeters of blood within the first minute after the end of the exercise. The ventilation was measured for each minute of the exercise and for each of the succeeding five minutes. The value in the table for "ventilation after exercise" refers to the ventilation during the minute in which the blood was obtained, while the value for resting ventilation is the average of the five minute fore-period.

In all subjects the first exercise performed was slight, almost equivalent to walking at a slow pace. Severe exercise was usually not done by the patients, but several of the normals repeated the performance at a faster rate (one "round-trip" over the platform and back in seven and one half seconds) this being equivalent to rapid walking. Immediately after this exercise a third blood sample was taken.

In this technique of obtaining venous blood three points are emphasized:

- (a) There was absolutely no stasis present during the sampling.
- (b) No pain was experienced when the sample was drawn. For this reason psychic factors due to pain can be excluded. In this entire study whenever the subject was disturbed by pain incidental to blood sampling, or when there was any evidence of apprehension or other psychic disturbance the experiment was abandoned.
- (c) The arm did not partake of the exercise.

### *Results*

*Ventilation.* That the exercise employed in this series is sufficient to markedly increase the ventilation is evident from the tables. The normals increased their ventilation from 18 to 104 per cent while the patients increased their ventilation from 32 to 121 per cent. This increase in both groups is sufficiently great so that chemical changes of sufficient magnitude to account for it should be detectable.

*Oxygen saturation.* The range of oxygen unsaturation in volumes per cent  $O_2$  i.e., the  $O_2$  utilized during capillary flow varies over the same range in the cardiac patient as in the normal both at rest and following exercise. The average increase for exercise (Table 1) for the normals was only 0.5 volume per cent  $O_2$  and for the patients only

TABLE I

The ventilation and acid base condition of venous blood of the arm at rest and after mild exercise

Subject	Date	Diagnosis	Control period—rest			After mild exercise			Change from resting value after mild exercise											
			Vital capacity	O <sub>2</sub> capacity	B <sub>134</sub>	O <sub>2</sub> saturation	Serum CO <sub>2</sub> content	pH	CO <sub>2</sub> excretion	O <sub>2</sub> saturation	Serum CO <sub>2</sub> content	pH	Ventilation increase per minute per square meter	O <sub>2</sub> unsaturation	Serum CO <sub>2</sub> content	pH				
J 1000			liters	liters per cent	liters	liters per cent	mm Hg	per cent	liters per cent	liters per cent	mm Hg	per cent	liters per cent	liters per cent	mm Hg	pH				
T R H Nov 10	Normal		0	2.57	18.00	3.46	67.4	62.5	35.7	7.50	6.41	4.56	61.4	32.1	7.54	2.95	+2.18	-1.1	-3.6	+0.04
J A C Oct 29	Normal		0	2.28	17.65	3.07	41.8	67.1	40.9	7.47	6.27	4.07	67.6	37.7	7.51	3.20	+0.11	+0.5	-1.1	+0.04
G E C Oct 22	Normal		0	1.99	18.58	3.75	6.06	68.2	43.5	7.45	6.61	8.23	69.7	43.5	7.46	2.86	-2.17	+1.5	0	+0.01
W E W Oct 21	Normal		0	2.38	19.10	4.28	4.45	66.5	38.8	7.49	6.23	5.42	66.1	38.5	7.49	1.95	-0.97	-0.4	-0.3	0
E J Oct 21	Normal		0	2.18	16.88	3.07	4.23	67.6	47.1	7.41	4.65	5.93	68.8	42.8	7.46	1.58	-1.70	+1.2	-4.8	+0.05
J T J Nov 18	Syphilitic aortic insufficiency	+	1.45	16.05	4.72	5.15	63.4	37.0	7.49	10.45	5.27	63.8	37.2	7.49	5.73	-0.12	+0.4	+0.2	0	
L C Nov 3	Hypertension	+	1.34	13.88	3.54	5.15	62.8	38.3	7.48	6.80	5.15	64.9	38.7	7.49	3.26	0	+2.1	+0.4	+0.01	
A C Oct 31	Hypertension	+	1.63	16.55	6.31	6.73	75.0	45.7	7.47	8.16	8.31	77.6	46.2	7.48	1.85	-1.58	+2.6	+0.5	+0.01	
A B Nov 12	Syphilitic aortic insufficiency	++	1.59	14.12	4.98	2.97	63.7	38.0	7.48	7.53	5.17	61.8	34.0	7.50	2.55	+2.20	-1.9	-4.0	+0.02	
R J Nov 6	Hypertension	++	1.59	14.85	3.38	6.37	7.20	42.0	7.49	7.48	5.64	71.7	40.9	7.50	4.10	+0.73	-0.3	-1.1	+0.01	
W C Oct. 28	Hypertension	++	1.27	15.10	4.40	5.65	60.3	32.2	7.53	9.30	7.83	62.0	33.9	7.52	4.90	-2.18	+1.7	+1.7	-0.01	

\* See text

TABLE 2

*Change from resting value produced by moderate exercise*

(Six of the experiments with mild exercise reported in Table 1 were continued with moderate exercise resulting in the changes tabulated in this table)

Subject	Ventilation increase per minute per square meter <i>liters</i>	Oxygen unsaturation <i>volumes per cent</i>	Serum CO <sub>2</sub> content <i>volumes per cent</i>	CO <sub>2</sub> tension <i>mm Hg</i>	pH
T R. H	5.42	+2.79	-1.9	-3.3	+0.03
J A C	4.17	+1.33	-3.2	-1.1	+0.02
G E C	7.21	-3.64	+2.0	+1.8	0
W E W	4.96	-	-2.8	+0.9	-0.03
L C	9.14	+0.42	+1.9	+3.8	-0.03

0.9 volume per cent. The results for oxygen utilization afford no adequate explanation for the marked increase in ventilation.

*Carbon dioxide content of the serum.* The range for the patients was greater than for the controls but all values were within normal limits. The mean values were almost exactly the same for the two groups. It is evident that these patients showed no evidence of diminished alkaline reserve, and hence that the difference in ventilation cannot be ascribed to changes in bicarbonate content.

As a matter of fact, in review of the eleven observations, the CO<sub>2</sub> content was slightly *greater* after exercise and the average figures were an *increase* of 0.3 volume per cent for the normal and 0.8 volume per cent for the cardiac.

*Hydrogen ion concentration of the serum.* At rest the values were on the average slightly more alkaline in the patients than in the controls, but here again overlapping occurred and with one exception all were within the normal limits of pH, reported for normal individuals in Nashville by Earle and Cullen (1929). After mild exercise four of the five normal subjects had an increase in pH, the fifth showing no change. Of the six patients, four had a slight rise in pH, one a slight decrease and one no change. After mild exercise the pH was on the average 0.03 more alkaline in the normals and 0.01 more alkaline in the patients than in the control determinations. After moderate exercise the normal subjects were often slightly more alkaline—average 0.01—than in the control period.

From these results it is clear that such differences in pH as were found after exercise must be looked on as *effects* rather than causes of the changes observed in ventilation.

*Carbon dioxide tension of the serum.* The resting values were usually somewhat lower in the patients, the average being 39 mm as opposed to 41 mm in the normals. However, two of the patients had values above the average for the normals and one of the controls had a value below the average for the patients. After mild exercise the carbon dioxide tension of the normal subjects was decreased in three instances and practically unchanged in two. In the patients the carbon dioxide tension was decreased in two, increased in one, and practically unchanged in three observations. (Differences of less than 1.0 mm are almost certainly not significant as an error of 0.2 volume per cent in carbon dioxide content plus an error of 0.02 in pH would cause a difference of approximately 2 mm in the calculated carbon dioxide tension. However, since the above errors are maximal for the methods used, changes of more than one millimeter are possibly significant.) The average change was -2.3 mm in the normal subjects, and -0.4 mm in the patients. After moderate exercise the carbon dioxide tension returned almost to the resting level.

It can therefore be stated that insofar as conclusions can be drawn from the venous blood of the arm, neither the increased ventilation after exercise in both the normal and diseased subjects, nor the increased ventilation in the latter as compared to the former under similar conditions can be attributed to changes in carbon dioxide tension. On the contrary such changes in the latter function as have been found are probably to be considered as results of increased ventilation.

#### B *The findings in arterial blood (Table 3)*

*Plan.* The observations of Haldane (1922) of Winterstein (1911), and of Hasselbalch (1912) have indicated the great importance of the hydrogen ion concentration of the arterial blood in the control of respiration. Other authors, Hooker Wilson, and Connell (1917), and Scott (1918), have believed that the arterial carbon dioxide tension has a more or less specific function in respiratory control. Since it is possible that the absence of any definite changes in venous blood from the arm might have been due to the buffering action of the muscles of the arm it seemed necessary to study arterial blood.

TABLE 3  
*The ventilation and acid base condition of arterial blood of the arm at rest and after mild exercise*

Diagnosis	Degree of cardiac disease*	Control period—rest				After mild exercise				Change from resting value								
		Vital capacity per square meter	O <sub>2</sub> capacity	Ventilation per minute per square meter	O <sub>2</sub> saturation	Serum CO <sub>2</sub> content	CO <sub>2</sub> tension	pH	Ventilation per minute per square meter	O <sub>2</sub> saturation	Serum CO <sub>2</sub> content	CO <sub>2</sub> tension	pH	Ventilation change per minute per square meter	O <sub>2</sub> saturation per cent	Serum CO <sub>2</sub> content per cent	CO <sub>2</sub> tension mm Hg	pH
Hypertension	±	1.91	18.75	2.60	91.8	63.4	33.9	7.53	7.64	96.9	62.5	35.0	7.48	5.04	+5.1	-0.9	+1.1	-0.05
Hypertension	+	1.76	17.92	4.30	94.6	69.2	40.4	7.49	9.56	98.7	69.1	41.1	7.48	5.26	+4.1	-0.1	+0.7	-0.01
Hypertension	+	1.72	16.60	5.04	91.3	68.8	39.3	7.50	6.57	97.8	68.9	38.4	7.51	1.53	+6.7	+0.1	-0.9	+0.01
Arteriosclerosis		1.74	17.80	3.44	96.0	55.4	31.6	7.50	9.03	100.0	55.1	32.2	7.49	5.59	+4.0	-0.3	+0.6	-0.01
Hypertension	++	1.76	16.95	4.10	93.7	63.0	30.1	7.58	6.94	99.3	64.4	32.2	7.56	2.84	+5.6	+1.4	+2.1	-0.02
Hypertension	++	1.54	16.82	4.70	93.0	58.0	27.1	7.59	9.03	95.7	56.1	27.9	7.56	4.33	+2.7	-1.9	+0.8	-0.03
Syphilitic aortic insufficiency	++	1.62	16.95	4.62	92.1	58.8	28.2	7.57	9.41	97.3	58.4	31.2	7.53	4.79	+5.1	-0.4	+3.0	-0.04
	1.28	18.12	5.52	92.9	60.9	34.0	7.51	9.10	98.8	58.1	33.1	7.50	3.58	+5.9	-2.8	-0.9	-0.01	
	1.84	18.75	4.86	96.3	57.9	33.0	7.50	10.98	98.8	55.8	29.2	7.54	6.12	+2.5	-2.1	-3.8	+0.04	

The experimental procedure was the same as that previously described except that brachial arterial punctures were done before and immediately after the exercise. The punctures were made with local anesthetic, novacaine. Whenever there was any pain or any difficulty in obtaining any sample the experiment was abandoned. Blood usually appeared in the syringe within twenty seconds after the end of the exercise and in most instances the entire sample was obtained during the first minute of the after period. The values for ventilation after exercise in the tables are those for the minute during which the blood samples were drawn.

The *oxygen capacity* of the blood was usually determined only once, a mixed sample consisting of equal parts of the blood drawn before and that drawn after exercise being used. In the one experiment in which capacity was determined both before and after exercise an increase of approximately one volume per cent was found. This is in agreement with the findings of Harrison, Robinson and Syllaba (1929), who reported increase in oxygen capacity during exercise.

The *oxygen saturation* varied between 90 and 100 per cent, all values being therefore within, or nearly within, normal limits. In most instances a small rise in oxygen content was noted after exercise. Similar results were obtained by Himwich and Loebel (1927) on normal subjects. It is evident that the changes in arterial saturation are the results rather than the cause of the increase in ventilation produced by exercise.

It was noted in the previous paragraph that pooled blood was used for the determination of the oxygen capacity. Since the oxygen capacity is greater after exercise, the effect is to increase the apparent oxygen saturation in exercise blood and to decrease it in resting blood. This effect is negligible in these experiments, but should be considered in using these data for absolute values.

The *carbon dioxide content of the serum* was unaltered (change of less than 0.2 volume per cent) twice increased once and decreased six times. All changes were of relatively small degree. The average for the nine observations was a diminution of 0.8 volume per cent. Such a change could be explained either by a slight increase in non-volatile acid or by over-ventilation.

The *hydrogen ion concentration of the serum* was unchanged after excess exercise (change of 0.02 or less) in five instances, decreased in

three and increased in one. The mean change was a decrease of 0.01 pH which is almost the average error of the method. It is to be noted that in the resting cardiac patients there is a definite tendency toward alkalinity. This is in agreement with the observations of Fraser, Harris, Hilton and Linder (1928).

The carbon dioxide tension was not appreciably altered in five instances (change of less than one millimeter) was increased in three and decreased in one. The average change was an increase of 0.3 mm which is much less than the error of the method.

It may be noted that there was on the average a very slight shift toward acidity as regards carbon dioxide tension and in respect to hydrogen ion concentration. These changes were so slight that in six of the nine observations, they might have been considered in any given instance as due to error. However, the question arises could such slight changes account for the observed change in ventilation, which was usually increased by more than fifty per cent and often was more than double the resting value? Expressed otherwise this question becomes Is the respiratory center sensitive to changes in carbon dioxide tension or hydrogen ion concentration of such small magnitude as to be scarcely detectable by our present methods? As Gesell (1925) has pointed out, that theory of respiratory control which assumes that the hydrogen ion concentration of the arterial blood is the sole or chief factor in respiratory control necessitates the assumption that such an extreme sensitivity exists. Hence, one might assume that increase in acidity of the arterial blood was the cause of the increased ventilation observed in our patients after exercise. If this be true one has to assume first that in four instances a decrease in pH of less than 0.02 was sufficient increased acidity to stimulate the center and secondly that the two cases of increased pH were due to errors in technique. Since changes in blood pH can only affect the center by resultant changes in the center itself as has been emphasized by Gesell (1925), it seems extremely unlikely that the increased ventilation in these instances was due to the changes in blood pH. However, additional attempts were made to determine the relative sensitivity of the breathing to change in arterial pH. The well known fact that ammonium chloride causes an acidosis was utilized for the following experiment.

*C Observations on arterial blood before and after the administration of ammonium chloride*

The usual measurements of ventilation and the usual analysis of arterial blood were made on two patients in the fasting resting state. They were then given ammonium chloride by mouth, two grams every half hour for four doses and the observations were repeated. The data are seen in Table 4. Their oxygen saturations decreased, probably due to a lowering of the oxygen dissociation curve. In both patients the carbon dioxide content diminished markedly, the pH decreased and the carbon dioxide tension was also reduced. It should be noted that the decline in pH was greater than that found after the mild exercise of this study. The ventilation of both patients increased very slightly—to a much less degree than that which occurred after the exercise. Neither patient complained of any dyspnea, in fact, both volunteered the information that they felt better after taking ammonium chloride. We are forced to conclude from these observations that the respiratory center is much less sensitive to changes in hydrogen ion concentration than has been generally believed to be the case. These experiments also indicate that the slight shift toward acidity which was found in the arterial blood of some—not all—of our patients after exercise can in no sense be regarded as the cause of the marked increase in ventilation during and after exercise.

*D Time curves of the gases in arterial blood before, during,  
and after exercise*

It seemed possible that blood samples obtained after exercise might not represent the state of the blood during exercise, and hence that the increase in ventilation might have been due, in the first instance, to chemical changes which were not detectable in blood drawn two to three minutes after the beginning and one half to one minute after the end of the exertion. It might be argued that there was an immediate increase in either hydrogen ion concentration or CO<sub>2</sub> tension sufficient to stimulate respiration and that this increased respiration caused enough over ventilation to bring the pH back to, or above, its initial level. The only answer to this argument appeared to lie in studying the blood continuously. The subject lay in bed. Resting ventilation was measured. The needle was then inserted in the brachial artery.

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It may be noted that there was on the average a very slight shift toward acidity as regards carbon dioxide tension and in respect to hydrogen ion concentration. These changes were so slight that in six of the nine observations, they might have been considered in any given instance as due to error. However, the question arises could such slight changes account for the observed change in ventilation, which was usually increased by more than fifty per cent and often was more than double the resting value? Expressed otherwise this question becomes Is the respiratory center sensitive to changes in carbon dioxide tension or hydrogen ion concentration of such small magnitude as to be scarcely detectable by our present methods? As Gesell (1925) has pointed out, that theory of respiratory control which assumes that the hydrogen ion concentration of the arterial blood is the sole or chief factor in respiratory control necessitates the assumption that such an extreme sensitivity exists. Hence, one might assume that increase in acidity of the arterial blood was the cause of the increased ventilation observed in our patients after exercise. If this be true one has to assume first that in four instances a decrease in pH of less than 0.02 was sufficient increased acidity to stimulate the center and secondly that the two cases of increased pH were due to errors in technique. Since changes in blood pH can only affect the center by resultant changes in the center itself as has been emphasized by Gesell (1925), it seems extremely unlikely that the increased ventilation in these instances was due to the changes in blood pH. However, additional attempts were made to determine the relative sensitivity of the breathing to change in arterial pH. The well known fact that ammonium chloride causes an acidosis was utilized for the following experiment.

and a resting sample of blood was obtained. The syringe was then detached from the needle which was held *in situ* in the artery. Exercise, consisting of alternately flexing and extending the opposite thigh and leg was then performed. This exercise was continued for two minutes at a rate of thirty complete movements—flexion and extension—per minute. During each of these two minutes and also during the first minute after the work had ceased successive blood samples were obtained.

Four such observations were made on three patients. The findings are shown in Table 5. In none of the patients were significant reductions in the pH or increases in carbon dioxide tension observed. Such slight changes as did occur in these functions were more often in the direction of increased alkalinity, but most of the changes were within the limit of error of the methods used. However, ventilation was increased in every case.

The changes noted in the arterial saturation, although of slight degree, are of some interest. There was a tendency toward slight diminution during, and slight increase after, exercise. A possible explanation is as follows. During exercise the cardiac output probably increases relatively soon, when the pumping action of the muscle begins. However, the ventilation increases more slowly, being always greater during the second than during the first, minute of exercise. At the cessation of effort the pumping action of the muscles ceases and a rapid diminution in venous return and in cardiac output probably occurs. However, the ventilation remains elevated for a longer time. At the time when the increase in circulation is relatively greater than that in ventilation, one would expect a tendency for the arterial oxygen to decrease whereas when the reserve was true, an increased saturation would be expected.

In any case the changes in oxygen, like those in carbon dioxide and hydrogen ion concentration are much too small and too inconstant to account for the changes in ventilation.

## DYSPNEA OF EXERTION

TABLE 4  
*The effect of ammonium chloride on ventilation and on acid-base condition of arterial blood*

Subject and chief diagnosis	Date	Vital capacity per square meter	Time at which blood was taken	Ventilation per minute per square meter	O <sub>2</sub> capacity	O <sub>2</sub> content	O <sub>2</sub> saturation	Serum CO <sub>2</sub> content	CO <sub>2</sub> tension	pH	Remarks
E G Syphilitic aortic insufficiency Cardiac failure++	1080 December 20	liters 1.86	Before NH <sub>4</sub> Cl  After 8 grams NH <sub>4</sub> Cl	liters 4.04  4.14	volumes per cent 14.28  14.05	volumes per cent 98.4  89.6	per cent 14.53  89.6	volumes per cent 61.9  48.6	mm Hg 36.9  33.8	7.48  7.41	No dyspnea following NH <sub>4</sub> Cl
A C Syphilitic aortic insufficiency Cardiac failure++	1087 December 20	liters 1.87	Before NH <sub>4</sub> Cl  After 8 grams NH <sub>4</sub> Cl	liters 4.54  4.84	volumes per cent 15.74  18.40	volumes per cent 94.7  90.8	per cent 58.4  45.4	volumes per cent 30.1  26.6	mm Hg 7.53  7.49	No dyspnea following NH <sub>4</sub> Cl	

TABLE 5 (continued)

Sub- ject	Chief diagnosis	Date	Degree of cardiac disease*	O <sub>2</sub> capacity volume per cent	Time of blood specimen	O <sub>2</sub> content volume per cent	Serum CO <sub>2</sub> content per cent	CO <sub>2</sub> tension mm. Hg	Ventilation per minute per square meter	Vital capacity per square meter	Hours
A. C.	Syphilitic aortic insufficiency	1930 December 24	++	17.92	Before exercise During 1st minute of ex ercise During 2nd minute of ex ercise 1st minute after exercise	16.70 16.70	93.2 93.2	33.3 33.3	7.49 7.49	4.76 5.89	1.97
W. C.	Hypertension Arterio- sclerosis	December 27	+	18.28	Before exercise During 1st minute of ex ercise During 2nd minute of ex ercise 1st minute after exercise	17.50 16.88	95.7 92.4	58.3 58.2	7.49 27.2	6.68 7.59	1.82

## DYSPNEA OF EXERTION

TABLE 5  
*Time relationship of exercise and acid-base condition of arterial blood*

Subject	Chief diagnosis	Date	Degree of cardiac disease*	O <sub>2</sub> capacity volumes per cent	Time of blood specimen	O <sub>2</sub> content volumes per cent	O <sub>2</sub> saturation per cent	Serum CO <sub>2</sub> content mm Hg	CO <sub>2</sub> tension mm Hg	pH	Ventilation per minute per square meter	Vital capacity per square meter liters
P II	Asthma Emphysema Syphilitic aortic insufficiency	1950	++	18.27	Before exercise	16.34	89.5	55.2	29.2	7.55	4.46	1.65
		December			During 2nd minute of exercise	15.85	86.8	54.8	28.7	7.54	7.33	
		23			1st minute after exercise	16.95	92.9	55.2	28.9	7.54	5.68	
	December 26	1950	++	16.35	Before exercise	15.36	94.0	51.4	28.7	7.51	5.66	1.56
					During 1st minute of exercise	15.36	94.0	51.1	28.5	7.51	7.40	
					During 2nd minute of exercise	15.36	94.0	51.0	27.8	7.52	8.06	
					1st minute after exercise	15.50	94.8	51.0	29.1	7.50	6.82	

\* See text

TABLE 6  
Ventilation and acid-base condition of blood from internal reader at rest and after mild exercise

Subject	Date	Diagnosis	Control period—1950				After mild exercise				Change from resting period			
			Vital capacity, liters	O <sub>2</sub> capacity, liters	Ventilation, liters per minute per square meter	Serum CO <sub>2</sub> tension, mm. Hg	Ventilation, liters per minute per square meter	O <sub>2</sub> unsaturation	Serum CO <sub>2</sub> tension, mm. Hg	Vital capacity, liters	Ventilation, liters per minute per square meter	O <sub>2</sub> unsaturation	Serum CO <sub>2</sub> tension, mm. Hg	pH
E. V.	1950 December 9	Hypertension	2.01	1.76	5.30	59.8	7.02	67.0	38.2	7.50	10.16	56.2	7.50	7.02
E. G.	1950 December 5	Syphilitic aortic insufficiency	1.51	1.67	4.58	54.4	7.62	70.2	42.7	7.47	8.30	54.4	7.62	70.2
R. J.	December 8	Hypertension	1.70	1.671	4.12	62.4	6.29	69.0	40.3	7.49	6.37	69.6	5.08	69.6

\* See text.

*E The findings in the blood from the internal jugular vein*

As a result of the observations which have been given it is evident that the increase in ventilation brought on by exercise cannot be explained by changes in the chemical composition of the arterial blood or of the venous blood from the arm. However there remains the possibility that decreased blood flow through the brain might be present and account for the dyspnea. Is cardiac dyspnea due to decreased blood flow through the brain? Some authors seem to think so. Means (1924) ascribed cardiac dyspnea to diminished output of the heart, and presumably meant more specifically, decreased cerebral blood flow. Meakins and Davies (1925) discuss the complicated nature of cardiac dyspnea, and they, too, are inclined to regard decreased blood flow as being the most important factor. These conclusions have been, in each instance, based on very indirect evidence. Gesell (1925) has demonstrated the great importance of the blood flow through the brain in the control of respiration. But is the flow of blood through the brain decreased in patients with cardiac dyspnea?

In order to obtain evidence on this point studies were made on the blood from the internal jugular vein. This was obtained according to the technique described by Myerson, Halloran and Hirsch (1927) and by Lennox (1930). Otherwise, the experimental procedure was exactly the same as that already described. Observations were made before and after the mild exercise described under "methods" on three subjects and in each of them the taking of the second jugular sample was completed within the first one and one-half minutes after exercise. The data are shown in Table 6.

The oxygen content after exercise was the same as at rest in one patient, rose in another and fell in the third. Values for mean unsaturation were 6.98 and 6.81 volumes per cent for rest and exercise respectively. These values are close to the average of 7.3 volumes per cent unsaturation found by Lennox (1930) in the internal jugular blood of sixty patients without cardiac or pulmonary disease. It is therefore evident that, unless one is willing to make the unlikely assumption of diminished metabolic rate in the brain during exertion, the dyspnea brought on by mild exercise in patients with cardiac disease cannot be attributed to decrease in cerebral blood flow.

TABLE 7  
*The effect of maximal exercise on the acid-base conditions of arterial blood*

Subj- ject	Date	Diagnosis	Degree of cardiac disease*	O <sub>2</sub> capacity per cent	Time of blood sample	O <sub>2</sub> content	O <sub>2</sub> saturation per cent	Serum CO <sub>2</sub> tension	pH	Exercise performed	Remarks
J P J											
G C	January 10	Normal	0	20.70	Before exercise 1st minute after exercise	19.60 20.70 20.70	94.7 100	32.1 60.2 41.5	7.53 7.39	Standing run ning 220 high steps per min ute for two minutes	Maximal exercise
R T	January 10	Normal	0		Before exercise 2nd minute after exercise					Standing run ning 220 high steps per min ute for two minutes	Maximal exercise
E C	January 8	Syphilitic aortic insufficiency	+	15.75	Before exercise 1st minute after exercise	14.30 14.78	90.4 93.9	33.1 53.8	7.55 7.46	Stair climbing up and down 48 steps per minute for two minutes	Maximal exercise
A C	January 7	Syphilitic aortic insufficiency	++	18.06	Before exercise 1st minute after exercise	17.10 17.34	94.7 95.8	59.8 58.7	7.55 7.51	Standing run ning 180 low steps per min ute for two minutes	Severe exer- cise almost maximal

\* See text.

*The carbon dioxide content* did not undergo significant changes and this is further evidence against diminished cerebral circulation. The pH was unchanged in one patient and increased slightly in the other two. The tendency was for the blood to be more alkaline after exercise but the degree of change was too slight to be significant. The *carbon dioxide tension* was less after exercise in two patients and unchanged once. Such small changes as did occur in the acid-base state of the blood were in the direction of increased alkalinity and are therefore to be regarded as effects rather than causes of the increased ventilation.

*F Changes in the arterial blood of normal subjects and patients with cardiac disease before and after maximal exercise*

In order to determine whether patients with cardiac disease are able to exercise sufficiently severely to become acidotic to any marked degree, observations were made before and immediately after an exertion which was as severe or nearly as severe as the subject could perform. In these instances ventilation was not measured as it was felt that the face mask might hinder the respiration and hence limit the severity of the exercise. As can be seen in Table 7, the arterial saturation increased. The changes in carbon dioxide content and in hydrogen ion concentration were much less marked in the patients than in normal subjects who performed much severer exercise but had about the same degree of distress. Despite relatively striking diminution in pH and carbon dioxide content, the carbon dioxide tensions were only slightly affected by the severe exercise.

As the patients were doing all, or nearly all, they could do, it seems fair to conclude that individuals with cardiac failure are unable to perform severe enough exercise to markedly affect their acid-base balance. *Their dyspnea checks them before acidosis has become severe*.

In view of this finding certain conclusions drawn in a previous paper in this series (Harrison and Pilcher (1930)) must be revised. It was found that patients with cardiac failure are unable to exert themselves sufficiently severely to acquire a large oxygen debt. Believing that the limiting factor of exercise in such patients, as in normal subjects, was the dyspnea due to the rise in hydrogen ion concentration of the blood, Harrison and Pilcher, who did not study the blood, assumed that their patients had, on the performance of relatively slight exercise, and hence following the production

TABLE 7  
*The effect of maximal exercise on the acid-base conditions of arterial blood*

Subject	Date	Diagnosis	Degree of cardiac disease*	O <sub>2</sub> capacity columns per cent	Time of blood sample	O <sub>2</sub> content molecules per cent	O <sub>2</sub> saturation Air cont. per cent	Serum CO <sub>2</sub> content Air cont. per cent	CO <sub>2</sub> tension mm. Hg	pH	Exercise performed	Remarks
G C	January 10	Normal	0	20.70	Before exercise 1st minute after exercise	19.60 20.70	94.7 100	60.2 41.5	32.1 30.2	7.53 7.39	Standing running 220 high steps per minute for two minutes	Maximal exercise
F T	January 10	Normal	0		Before exercise 2nd minute after exercise			58.1 32.6	27.6 27.0	7.50 7.33	Standing running 220 high steps per minute for two minutes	Maximal exercise
E G	January 8	Syphilitic aortic insufficiency	+	15.75	Before exercise 1st minute after exercise	14.30 14.78	90.4 93.9	64.7 53.8	33.1 33.5	7.55 7.46	Stair climbing up and down 48 steps per minute for two minutes	Maximal exercise
A C	January 7	Syphilitic aortic insufficiency	++	18.06	Before exercise 1st minute after exercise	17.10 17.34	94.7 95.8	59.8 58.7	30.5 32.8	7.55 7.51	Standing running 180 low steps per minute for two minutes	Severe exercise almost maximal

\* See text.

of a relatively small amount of lactic acid, a marked acidosis of the blood stream. From this they concluded that the buffering power of the tissues was diminished in such patients. However, the present work shows that one of their premises was incorrect, because the limiting factor in exercise in patients with cardiac failure is not acidemia. Hence the inability of the patient with cardiac failure to acquire a large oxygen debt can no longer be considered as indicative of diminution in muscle buffers. (The other evidence for diminished muscle buffers is of course not affected by this study. It may be also said that the findings in the present work are in no sense contrary to the data of Harrison and Pilcher, but only to the conclusions which they drew.)

#### DISCUSSION

*Application of data to cardiac disease.* At the beginning of the paper it was stated that our main object was to attempt to ascertain (1) why patients with cardiac disease usually have a greater ventilation than normal individuals upon the performance of a given exercise and (2) how decreased vital capacity tends to produce dyspnea. From the foregoing data it seems clear that the present researches have failed entirely to furnish an adequate answer to either question. All of our findings point toward the conclusion that such small changes as do occur in the composition of the blood are usually to be considered as effects rather than as causes of increased ventilation. The absence of any consistent alteration in the blood also seems to indicate that diminution in vital capacity predisposes to dyspnea by some mechanism other than changes in the oxygen saturation or acid-base condition of the blood. At the present time we are unable to state what this other mechanism may be. Studies on the subject are in progress.

The observations which have been made confirm those of Peters and Barr (1921), Eppinger, Kisch and Schwarz (1927), and Fraser, Harris, Hilton and Linder (1928). These investigators have reported similar more or less negative results in their attempt to correlate cardiac dyspnea with changes in blood composition. They found increased acidity occasionally and increased alkalinity more frequently. Most of their values were within normal limits. Fraser and his co-workers, having found normal or slightly alkaline values for the hydrogen ion concentration and the carbon dioxide tension of arterial blood in patients with cardiac dyspnea, concluded that the dyspnea was probably to be attributed to decrease in blood flow through the respiratory center and

postulated alterations in the blood draining the brain. Our finding jugular blood seem to invalidate this hypothesis. It must be admit that an adequate explanation for cardiac dyspnea is entirely lacking the present time. Such an admission seems to us to be a distinct forward. So long as it is assumed, on insufficient evidence, that symptoms of cardiac failure are essentially and primarily due to c stantly diminished cardiac output, the various problems of the sub are likely to be considered as solved and further progress in the sub will be delayed.

It is our belief that very little is known concerning the cause cardiac dyspnea.

Certain other features in connection with the present study may mentioned as requiring further elucidation. It is of some interes note that the venous blood (both jugular and cubital) usually beco slightly more alkaline after mild exercise, whereas the arterial bl less commonly exhibited this change. The carbon dioxide conten the venous blood of the arm was in the majority of instances slig greater after than before exercise, whereas, that of the arterial bl tended to be slightly less. These facts suggest that the blood buffered in some way as it passed through the tissues. Either a s of chloride from serum to cells or a passage of base from tissue to ser would explain the findings. Further observations on this point needed.

In a previous paper (Pilcher, Clarke and Harrison (1930)) exam of patients with cardiac failure and acidemia were presented should be remembered that our earlier studies, which dealt particula with edema were necessarily carried out on severely decompensa patients and several of them had diminished alkaline reserve of blood. As a result of those studies it was concluded that edema te to cause tissue acidosis by interfering with the diffusion of oxy. The fact that cardiac dyspnea of the type here studied is not essenti and primarily due to diminished blood alkalinity does not mean i acidosis never occurs in patients with cardiac failure. Patients w extensive edema may have diminution in the reserve alkali, low va for pH have been found in individuals dying from cardiac dist (Pilcher, Clark and Harrison (1930)) but as the present studies indic acidosis is usually only a very late manifestation of congestive faili

and cannot be regarded as the essential cause of dyspnea in patients with relatively early cardiac disease

*Application of data to general subject of respiration* Complete data for normal individuals was obtained only in the first series of experiments on venous blood from the arm. The evidence there obtained was that the chemical changes in the blood of the normal were not essentially different from those of patients with early cardiac disease.

This evidence and the cumulative data of both arterial blood and internal jugular blood from the brain in the subjects with early cardiac disease indicate (1) that the respiratory mechanism is not as sensitive to changes in acid-base condition as has been generally assumed and (2) that changes in the acid-base condition and oxygen saturation constitute only one factor in the control of respiration. This conclusion is substantiated more fully in the following report on orthopneic dyspnea (Calhoun, Cullen, Harrison, Wilkins and Tims (1931))

The results reported here and those found concurrently in Dills' laboratory (Myerson, Loman, Edwards and Dill (1931)) are in entire agreement. Dill and his associates studied simultaneously blood from the jugular vein, femoral vein and femoral artery during partial anoxemia and found no accumulation of lactic acid or other fixed acid. Blood from the brain was within the normal range in most subjects.

#### SUMMARY

Measurements of the ventilation, oxygen content, hydrogen ion concentration and carbon dioxide content of the blood before, after, and in a few instances during mild exercise have been made on patients with cardiac disease with relatively mild congestive failure and on normal subjects. Although the exercise was mild it was sufficient to cause slight subjective respiratory distress in the majority of the patients and to cause an increase of 50 to 100 per cent in ventilation. The following results have been obtained.

In *arterial blood* neither during nor immediately after mild exercise were there observed significant alterations in the hydrogen ion concentration, carbon dioxide content or carbon dioxide tension. The changes which occurred are to be interpreted as being, in the main, effects rather than causes of increased ventilation.

At rest the *venous blood* of the arm of the patients was almost identical with that of the normal subjects in regard to oxygen unsaturation, and

carbon dioxide content. As an average the pH was slightly higher and the carbon dioxide tension slightly lower in the patients, but the differences were too slight to be significant. After the mild exercise the blood of both groups tended to be slightly more alkaline and the oxygen unsaturation was slightly greater in the patients than in the normal subjects. Any chemical changes in the blood are to be regarded in both groups as effects rather than causes of the increase in ventilation.

In blood from the *internal jugular* of subjects at rest the oxygen unsaturation in the patients was slightly but not significantly less in the patients than the average values found by other investigators for jugular blood in normal subjects. After mild exercise the oxygen content was not altered. The carbon dioxide content, pH and carbon dioxide tension of the jugular blood were unchanged or slightly altered in the direction of alkalinity after exertion.

*Ammonium chloride* administered by mouth caused relatively great decrease in pH and carbon dioxide content of the arterial blood and relatively slight increase in ventilation, whereas mild exercise caused slight or no change in blood gases and marked increase in ventilation.

*Maximal exertion* in normal subjects caused marked reduction in carbon dioxide content and pH of arterial blood. In patients with cardiac failure maximal exertion which, although actually less than that performed by the normal subjects was accompanied by comparable respiratory distress, was attended by less marked reduction in carbon dioxide content and pH of the arterial blood. The limiting factor in exertion in such patients is not acidosis, but some other mechanism.

The respiratory mechanism is less sensitive to changes in acid base condition of the blood than has been generally believed. It appears that both in normal subjects and in patients with cardiac disease delicate respiratory adjustments to mild exercise are made quite independently of changes in the oxygen saturation and the acid base condition of the blood.

The observations do not lend support to the notion that the blood flow through the tissue is significantly less than normal in patients with slight cardiac failure either at rest or upon the performance of mild exercise. The findings are contrary to the idea that dyspnea in patients of this type is due to diminution in cerebral blood flow. Cardiac dyspnea is apparently due to some other, as yet unknown, cause.

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## STUDIES IN CONGESTIVE HEART FAILURE

### XIV ORTHOPNEA ITS RELATION TO VENTILATION VITAL CAPACITY OXYGEN SATURATION AND ACID-BASE CONDITION OF ARTERIAL AND JUGULAR BLOOD<sup>1</sup>

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#### INTRODUCTION

A number of hypotheses have been advanced as to the mechanism of the production of orthopnea. In general, these may be divided into three main groups.

1 *Diminished cerebral blood flow* Krehl (1916) believed that orthopnea, as well as other types of cardiac dyspnea, is always due to an inefficient interchange of gases between the blood and certain cells of the medulla. He pointed out that slowing of the blood stream was one of the means by which such a deficiency could come about. Sir James Mackenzie (1925) claimed that cardiac dyspnea of all types was primarily due to a deficient cardiac output. Neither supported their contentions by experiments. Recently, Ernstene and Blumgart (1930) investigated the subject and interpreted their studies as indicating that orthopnea was due to diminished cerebral blood flow. They stated

' This theory is based on the fact that increased cerebral venous pressure diminishes intracranial blood flow thereby favoring increased anoxemia of the respiratory center. In the upright position the pressure in the veins about the respiratory center is kept more nearly normal than in any other position and the blood flow in the capillaries feeding these veins is increased. In general it was found that the higher the venous pressure the greater was the orthopnea. When orthopneic patients were placed in the recumbent position with the head flat, simple elevation of the head by flexion of it on the thorax produced, almost without exception conspicuous diminution of the respiratory distress but had no significant effect on the

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vital capacity of the lungs      The extent of the diminution of the vital capacity of the lungs does not seem to be an important factor in determining the degree of elevation which gives the patient maximum relief Orthopnea of necessity was observed in patients in whom the vital capacity of the lungs was not significantly increased by changing from the recumbent to the sitting position "

2 *Deficient aeration of the blood in the lungs* Haldane, Meakins, and Priestley (1919) pointed out that the expansion of the lungs is uneven in the recumbent posture In normal subjects this is compensated for by slower and deeper breathing, but in patients with cardiac failure with diminished vital capacity, the depth of breathing cannot be materially increased Consequently, they believed that the arterial saturation with oxygen was less in the reclining position in such patients and that orthopnea was due to this fact

Meakins and Davies (1925) believed that decreased arterial saturation in the horizontal position was an important factor in the production of orthopnea Wilson (1928) confirmed Bohr's (1907) observation that the reserve air was greater in the sitting than in the recumbent posture Consequently, he believed that orthopnea could be explained by an assumption of decreased arterial saturation However, none of these authors performed analyses of arterial blood

3 *Mechanical changes in the lungs* Bohr, in 1907, showed that the vital capacity and the reserve air were greater in the sitting than in the recumbent position Rubow (1909) believed that kinking of the veins in the recumbent posture caused increased resistance in the pulmonary circulation Christie and Beams (1922) found reduction of vital capacity in the recumbent position The average degree of diminution was 5.5 per cent in two hundred and seventy normal subjects and 26.5 per cent in a series of seven patients with orthopnea They conclude that the decrease in vital capacity was the cause of the distress felt in the horizontal position Ernstene and Blumgart (1930) found much smaller difference in vital capacity in their orthopneic patients who had on the average only eight per cent greater vital capacity in the sitting than the recumbent position

Field and Bock (1925) found diminished cardiac output in the sitting as compared to the recumbent position, and believed that the respiratory distress which comes on lying down is due to pulmonary congestion and consequent diminution in vital capacity The most recent observations on the subject, those of Grollman (1928), did not demonstrate much change in cardiac output with change of posture

Blackhall-Morison (1928) believed that the orthopneic position produced benefit by limiting the venous inflow into the heart Hirschfelder (1913) thought that there were several different factors responsible for the relief experienced in the sitting posture These were (1) descent of the liver and diaphragm with consequent increase in the air space of the chest, (2) equali-

zation of the load on the two ventricles because of diminished venous return to the right auricle (3) diminished venous stasis in the medulla

#### *Present study*

The investigation of the relation of the *dyspnea of exercise* reported in the preceding paper (Cullen, Harrison Calhoun, Wilkins and Tims (1931)) in which it was found that the changes in the oxygen saturation and acid base condition of the blood were secondary to and not causative of the dyspnea indicates that similar relations might be true in *orthopnea*.

The concept that orthopnea in patients with cardiac failure is essentially due to decrease in cerebral blood flow seems extremely unlikely to us because it is contrary to the following well known clinical facts.

(1) Syncope, which is presumably due to decreased cerebral blood flow is often relieved by putting the head down.

(2) Orthopnea occurs in a variety of conditions such as congestive heart failure massive ascites pleural effusion, pneumothorax and some cases of pneumonia. All of these conditions are associated with decrease in vital capacity, but only one of them, namely, cardiac failure is regularly associated with increase in venous pressure. Is orthopnea in cardiac disease to be regarded as being entirely different from orthopnea in other conditions?

(3) Orthopnea is invariably absent in hemorrhage and shock which are known to be associated with diminution of the cardiac output.

(4) Orthopnea often occurs in patients with cardiac disease, who have no edema no enlargement of the liver, and no striking distension of the cervical veins.

In trying to evaluate the experimental data from which the various opinions reviewed here have been deduced, it became evident that more adequate observations were needed in which both the respiratory factors and blood chemical changes were studied in the same patients at the same time. The present paper reports the results of a study planned to furnish such data.

*Blood studies* Blood was obtained from the brachial artery and from the internal jugular vein, according to the technique described by Myerson, Halloran and Hirsch (1927) and by Lennox (1930). Analyses for oxygen and carbon dioxide were made, the hydrogen ion

concentration was determined and the carbon dioxide tension was calculated according to the methods described in the preceding paper of this series (Cullen, Harrison, Calhoun, Wilkins and Tims (1931) )

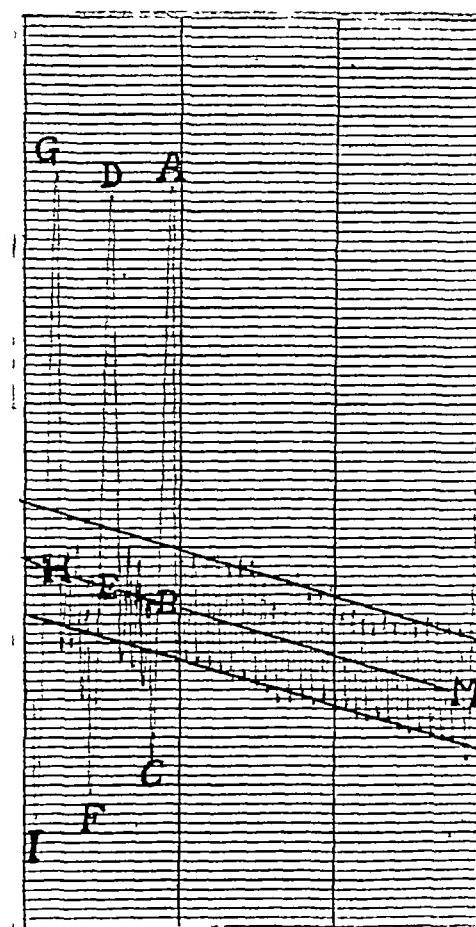


FIG 1

The curve runs from right to left. The upstroke denotes inspiration, the down stroke expiration. The line M-B-E-H represents the mid-position of the lungs. Three vital capacities were taken A-B-C, D-E-F and G-H-I. The values for these as calculated from the curve were 249 liters, 260 liters and 281 liters respectively. However, when the greatest of the three inspirations (A-B) is added to the greatest of the three expirations (H-I) the value obtained is 290 liters. It is obvious that this represents the true vital capacity and that the other values are all lower than the true, i.e., maximum vital capacity.

The observations were made on a group of eight patients suffering from different types and degrees of cardiac disease and congestive heart failure. The group includes one patient, J. L., with tuberculosis and asthma as representative of pulmonary complication. The findings in his case are summarized separately from those in the rest of the group. Three normal males were used as controls.

*Respiratory measurements* were made in the usual way. In measuring ventilation the patient breathed into a Tissot spirometer through a face mask equipped with appropriate valves. The vital capacity for some of the observations were obtained in the usual way but for the later determinations a refinement was introduced.

The subject was connected to a Benedict basal metabolism spirometer and allowed to breathe quietly for a few minutes, long enough to establish the slope of a mid-position line. The subject then made a series of maximal inhalations and exhalations. The deepest inhalation, measured from the mid position was added to the greatest expiration to establish the true maximum vital capacity. (See Fig. 1.)

## RESULTS

### A *The relation of orthopnea to the oxygen content and acid base balance of the blood*

The data are shown in Table 1. The findings in the case of J. L. are discussed separately at the end of this section. The *arterial oxygen saturation* was usually somewhat greater in the sitting than the recumbent position but the change was often slight and sometimes absent. Most of the values in either position were within normal limits. Consequently, it seems extremely unlikely that the benefit derived from the sitting position can be explained by the changes in arterial saturation, although in some cases this may be one factor.

*The jugular venous oxygen content* was less once, greater once and practically unchanged in the remaining four instances, in the sitting position as compared to the recumbent posture. The *arteriovenous oxygen difference of the blood passing through the brain* was determined in five instances and was in every case almost exactly the same in the two positions. However, the actual values varied widely in different persons the lowest being 3.62 and the highest 10.54 volumes per cent O<sub>2</sub>. The mean arteriovenous oxygen difference for the blood obtained from

TABLE I  
*The relation between acid-base condition of the blood and the ventilation in orthopnea*

Date	Degree of orthopnea	Position	Oxygen capacity	Arterial oxygen content	Jugular venous oxygen saturation	Arterio-venous oxygen difference	Carbon dioxide content of serum	pH of serum	Carbon dioxide tension of serum	Vital capacity, square meter	Ventilation per minute per square meter	Vital capacity ratio
				volumes per cent	volumes per cent	volumes per cent	mm Hg	mm Hg	mm Hg	liters		
December 3, 1930	Necessity	Recumbent	13.32									
		Sitting		7.99	7.02		7.40	7.46	31.7	1.21	6.88	5.68
December 12, 1930	Choice	Recumbent	19.12	17.44	91.3		61.5	7.53	28.2	1.51	7.14	1.73
		Sitting		18.75	98.1		58.7	7.52	32.8	1.56	5.68	3.61
December 16, 1930	Necessity	Recumbent	16.56	15.12	91.3		61.4	7.65	32.0	2.08	6.12	2.94
		Sitting		15.72	95.0		61.6	7.66				
December 9, 1930	Necessity	Recumbent	17.66	16.58	94.0			7.58		31.3	0.65	1.71
		Sitting		17.40	98.6		65.5	7.58		31.3	1.04	5.50
December 18, 1930	Choice	Recumbent	14.76	13.56	92.0	9.94	3.62	63.5	68.3	41.4	5.44	4.32
		Sitting		13.80	93.5	9.94	3.86	62.9	66.4	37.5	1.69	5.32
January 7, 1931	Necessity	Recumbent	18.40	12.00	65.2	8.37	3.63	73.9	78.6	45.5	63.2	3.15
		Sitting		12.36	67.1	8.61	3.75	72.9	78.0	7.34	1.06	3.60
January 16, 1931	Necessity	Recumbent	14.90	13.20	88.6	5.82	7.38	55.8	7.54	25.0	34.8	6.18
		Sitting		14.40	96.7	6.91	7.49	47.8	56.3	7.55	24.4	35.1
January 19, 1931	Necessity	Recumbent	16.24	14.80	91.1	6.92	7.88	71.8	75.5	7.47	36.6	4.76
		Sitting		14.55	89.6	6.92	7.63	70.4	79.3	7.56	35.2	47.3

the internal jugular vein was 6.51 volumes per cent in the recumbent position and 6.55 volumes per cent in the sitting posture. Therefore it is concluded that orthopnea cannot be due to diminished cerebral blood flow in the recumbent position. The only possible alternatives to this conclusion are the unlikely assumptions either that the portion of the brain drained by the jugular veins is different in the two positions or that the oxygen consumption of the brain is less when an individual lies down than when he sits up.

It may be noted that the average value for the utilization of oxygen in the blood passing through the brain of the patients with cardiac failure was almost exactly the same as those found by Lennox (1930) in his series of fifty-one individuals with no cardiac or pulmonary disease. His average value was 6.5 volumes per cent, but different normal subjects showed marked differences, as did our patients. The idea that cardiac dyspnea is primarily due to diminished cerebral blood flow has been widely accepted. These data, like those published in our preceding paper (Cullen, Harrison, Calhoun, Wilkins and Tims (1931)) fail to support this assumption. Some of our patients were severely decompensated but in only one of the five subjects (W J.) were the values for jugular oxygen much lower than the normal average. It seems clear that the cause of cardiac dyspnea must be sought elsewhere.

*The carbon dioxide content of the serum* was often slightly less in the arterial blood in the sitting than in the recumbent position. The difference was not striking, being usually less than one volume per cent and in three of the eight instances no change was noted. No constant difference in the carbon dioxide content of the jugular blood was observed. All values except one (W J.) were within normal limits.

*The hydrogen ion concentration* of the arterial serum was the same—within the limit of error of the method in the two positions in six of the eight observations. In one instance (W J.) decided shifts toward alkalinity were observed when the patient sat up. The pH of the blood from the internal jugular vein was the same in the two positions in three observations and more alkaline in the sitting position in three. The arterial pH was above 7.50 (both sitting and recumbent) in six of eight observations and in two of these it was above 7.60. The jugular blood was within normal range with one exception, W J., pH 7.59.

These findings are in accord with those of other investigators (Eppinger, Kisch and Schwarz (1927), Fraser, Harris, Hilton and Linder (1928)) that the majority of patients with cardiac insufficiency have blood which is toward or beyond the alkaline side of the normal range

The *carbon dioxide tension* of the arterial blood was practically the same in the two positions in six of eight determinations. In the remaining two subjects the carbon dioxide tension was lower sitting than recumbent. The carbon dioxide tension of the blood from the internal jugular vein was practically the same in the two positions in three patients, was somewhat less sitting in two individuals and was very much less sitting in one patient. The carbon dioxide tension in both positions was rather lower than normal in six of eight patients, and was normal twice.

It is evident from these data that the majority of patients with congestive failure have a slight or moderate alkalemia (tendency toward decreasing hydrogen ion concentration) from over ventilation, although the blood may be normal in regard to its alkali reserve. In none of the patients were the changes in the blood adequate to account entirely for the discomfort in the recumbent position. Their distress could be explained neither by deficient circulation nor by inadequate aeration, and such changes as were found in the blood were evidently effects rather than causes of their dyspnea.

The findings in J. L. are of special interest. This man had tuberculosis, emphysema, asthma and cardiac insufficiency. His ventilation was less than that of any other patient studied, and yet he had more subjective dyspnea than any other subject in the series. (It has been shown previously by Harrison, Turley, Jones and Calhoun (1931) that when respiratory obstruction is present the amount of ventilation is no index to the degree of subjective dyspnea.) He was the only patient of the series with marked anoxemia. The findings in the blood were those of uncompensated carbon dioxide excess and his distress was due to deficient aeration which was reflected in the blood. His carbon dioxide tension was extremely high and much higher lying than sitting. It is evident that, in this type of patient, orthopnea could be explained by the less efficient gas exchange in the lung in the recumbent posture.

*B Respiratory measurements in patients with orthopnea*

*Vital capacity and ventilation ratio* In order to compare quantitatively the respiratory embarrassment of the patients the values for the ratio  $\frac{\text{ventilation per minute}}{\text{vital capacity}}$  have been determined and are shown in Table 2.

Table 2 It has been shown previously (Harrison, Turley, Jones and Calhoun (1931)) that this quotient is a fairly accurate expression of the degree of respiratory distress. In the normal subjects the values are the same in the two positions and range between 1.50 and 1.80 whereas, the values for the patients are much higher, and in the majority of them the figures are considerably less in the sitting than in the recumbent posture. In an attempt to analyze the factors responsible for the greater dyspnea in the recumbent position the respiratory measurements are presented in detail in Table 2.

Observations were made of the ventilation per minute, respiratory rate and vital capacity of nine patients and of three normal subjects in the recumbent and sitting positions. The other data were calculated from these measurements. The total dead space (instrumental plus anatomical) was assumed to be 160 cubic centimeters. The mask used fitted the face closely and did not have a dead space of more than 30 cubic centimeters. The anatomical dead space was assumed to be 130 cc for each subject. The values for dead space ventilation and alveolar ventilation are therefore to be regarded as only crude approximations.

The order in which the various observations were made was varied, some of the subjects being studied first in the recumbent and then in the sitting position while in other individuals the observations while sitting were made first. For the sake of uniformity in tabulation the measurements in the recumbent posture are in each subject presented first.

The vital capacities of the patients were as would be expected, much less than those of the normal individuals. The latter had slightly greater vital capacity in the sitting position, the difference being about the same as that found by Christie and Beams (1922) in their large series of normal subjects. In all of the patients the vital capacity was greater in the sitting position and the degree of difference was not only relatively but in most cases was actually greater than in the normal.

TABLE 2

*Orthopnea in relation to respiratory measurements*

Subject	Diseases	Surface area	Date	Degree of orthopnea	Position	Vital capacity	Actual change in vital capacity	Change in vital capacity	Respiratory rate	Ventilation per minute	Mean tidal air	Vital capacity used per breath	Dead space ventilation per minute	Air convection per minute	Ventilation per square meter	Vital capacity ratio	Ventilation per minute
W E W	Normal	square meters 1.75	December 10, 1930	None	Recumbent	3.80	250	6.6	per cent per minute	liters	cc	per cent	liters	liters	liters	1.77	1.77
T R H	Normal	1.75	December 10, 1930	None	Recumbent	4.10	350	8.5	8	7.4	519	13.6	2.08	2.66	2.66	1.71	1.71
J A C	Normal	1.75	December 10, 1930	None	Recumbent	4.00	350	8.8	10	7.06	471	11.6	2.40	2.66	2.66	1.50	1.50
A I C	Syphilitic aortic insufficiency	1.66	December 12, 1930 January 2, 1931	Necessity Choice	Recumbent Sitting Recumbent Sitting	2.60 3.45 2.90 3.10	850 200 200 180	32.7 17 6.9 19	18 17 20 19	9.42 10.16 9.18 9.14	518 598 459 471	19.9 17.3 15.8 15.2	2.88 2.72 3.20 3.04	3.94 2.72 3.20 3.67	3.94 2.72 3.20 3.67	3.62 2.94 3.16 2.95	3.62 2.94 3.16 2.95
P H	Bronchitis Emphysema Hypertension	1.65	December 11, 1930 December 16, 1930	Necessity Severe Necessity	Recumbent Sitting Recumbent Sitting	1.50 2.05 1.78 2.15	550 370 20.8 26	36.7 26 28 9.40	32 26 28 26	13.48 11.08 8.24 9.40	421 426 294 362	28.0 20.8 16.5 16.8	5.12 4.16 4.48 4.16	5.06 4.20 2.28 3.18	5.06 4.20 2.28 3.18	9.00 5.41 4.63 4.37	9.00 5.41 4.63 4.37

TABLE 2 (continued)

Subject	Diagnosis	Surface area square meter	Date	Degree of orthopnea	Position	Vital capacity liters	Actual change in vital capacity cc.	Changes in vital capacity per cent	Respiratory rate per minute	Ventilation per minute liters	Mean tidal air cc.	Vital capacity used per breath per cent	Dead space ventilation per minute liters	Aireolar ventilation per minute liters	Ventral respiration per minute per square meter	Vital capacity ratio
J L	Tuberculosis Asthma Emphysema	1.54	January 7, 1931	Necessity	Recumbent Sitting	1.15 1.63	480 417	41.7 19	6.56 5.52	345 395	30.0 24.2	3.04 2.24	2.28 2.13	4.38 3.40		
D W	Hypertension	1.63	January 16 1931	Necessity	Recumbent Sitting	1.15 1.25	100	8.7	22 23	10.36 11.10	471 482	41.0 38.5	3.52 3.68	4.07 4.42	9.01 8.88	
E G	Syphilitic	1.69	December 18 1930	Choice	Recumbent Sitting	2.12 2.85	730	34.4	29 26	9.18 9.00	312 346	14.7 12.1	4.64 4.16	2.69 2.86	4.33 3.16	
W M J	Hypertension	1.98	January 17 1931	Necessity	Recumbent Sitting	1.90 2.20	300	15.8	14 14	9.44 8.62	657 616	34.6 28.0	2.24 2.24	3.64 3.22	4.96 3.92	
A B	Hypertension	1.67	January 15 1931	Choice	Recumbent Sitting	1.65 2.07	420	25.5	23 21	8.76 8.32	350 396	23.0 19.1	3.68 3.36	3.04 2.97	5.31 4.02	
W J	Hypertension Arteriosclerosis	1.63	January 23, 1931	Necessity Severe	Recumbent Sitting	1.50 1.85	350	23.3	32 21	16.00 11.92	500 568	33.3 30.5	5.12 3.36	6.71 5.25	10.68 6.44	
C M	Hypertension Emphysema	1.68	January 19 1931	Necessity	Recumbent Sitting	1.30 2.00	700	53.9	23 22	8.56 9.36	372 425	28.6 21.2	3.68 3.52	2.90 3.43	6.59 4.68	

orthopnea may breathe more rapidly in the recumbent than in the sitting posture while normal individuals may breathe slightly slower in the recumbent posture. In both positions the respiratory rate of the patients is usually faster than normal. It has also been shown that the tachypnea of patients with cardiac failure is not due to anoxemia or changes in the acid-base balance of the blood.

That the rate of breathing is closely related to reflexes from the lungs through the vagus nerves was demonstrated more than sixty years ago by Hering and Brauer (1868), who showed that distension of the lungs produced an expiratory movement, whereas, deflation of the lungs produced an inspiratory movement, these effects being absent when the vagus nerves were cut. These observations have been confirmed by numerous observers. It seems possible that if for any reason such as rigidity or congestion, air cannot enter certain parts of the lungs the remaining functioning portion might be distended sufficiently to excite the Hering Brauer reflex with much less air than is normally required and thus bring about an increased rate of breathing. Further data on the nervous control of respiration in relation to changes in the lungs are needed. In the succeeding paper of this series (Harrison, Calhoun, Cullen, Wilkins and Pilcher (1932)) such data will be presented.

Further, the amount of air required to distend a given functioning portion of a lung is more nearly a function of the "complementary air" (volume from mid-position of lung to maximum inspiration)—than of total vital capacity. If the rigidity or congestion which brings about reduced vital capacity is of such a nature that when a subject sits up the mid-position of the lung does not change proportionately as much as in the normal and there results an increased complementary air, one would expect that subject to breathe more slowly. In order to test this reasoning further observations were made. The recumbent subjects were connected to a Benedict spirometer. Vital capacity, complementary air and supplementary air were measured. The subject then breathed normally for three minutes during which the respirations were recorded. Then, without removing the mouthpiece, the subject sat up and the observations were repeated. After a few minutes rest the observations were again made, in this instance the measurements being taken first with the patient sitting. The data are shown in Table 3. The vital capacity in the sitting posture was slightly greater

TABLE 3

*The effect of posture on the mid position of the lungs the complementary air and the supplementary air \**

Subject and sex	Diagnosis	Position	Respiratory rate per minute	Complementary air cc.	Supplementary air cc.	Vital capacity cc.	Shift in mid position cc.	Degree of orthopnoea
F J F	Normal	Recumbent Sitting	14 14	2010 1720	730 1200	2740 2920	390	None
M B F	Normal	Recumbent Sitting	18 19	1820 1740	750 970	2570 2710	250	None
S S F	Normal	Recumbent Sitting	15 15	2530 2320	1270 1540	3800 3860	240	None
W E W M	Normal	Recumbent Sitting	15 15	2490 2280	1620 1990	4110 4270	250	None
T R. H M	Normal	Recumbent Sitting	7 8	2840 2630	1400 1630	4230 4260	180	None
W J M	Hypertension Arteriosclerosis Emphysema	Recumbent Sitting	24 25	1350 1140	790 1040	2140 2180	150	None
W C M	Hypertension Arteriosclerosis	Recumbent Sitting	18 17	1850 1850	460 700	2310 2500	150	Choice
L. H M	Bronchiectasis Asthma	Recumbent Sitting	14 13	2030 2120	410 580	2440 2700	220	Choice
A. C M	Syphilitic aortic insufficiency	Recumbent Sitting	23 23	910 1080	560 910	1470 1990	60	Necessity
M H F	Cardiac hypertrophy	Recumbent Sitting	39 32	520 660	480 580	1000 1240	190	Necessity

\* The terms 'complementary air' and "supplementary air" are used to denote the greatest amounts of air which can be inspired and expired respectively, from the mid position of the lungs

than in the recumbent in the normal subjects and in the patient who had no orthopnea, was moderately greater in the two subjects with orthopnea of choice, and was markedly greater in the subjects with severe orthopnea. The supplementary air was greater in all subjects in the sitting posture, the degree of increase being about the same in the patients as in the normal subjects. The complementary air was less in the sitting than in the recumbent posture in the normal subjects and in the patient with no orthopnea. In the orthopneic patients the complementary air was unchanged or greater in the sitting posture. The shift in the mid-position of the lungs on change of posture was usually greater than the change in vital capacity in the normal subjects, but the reverse was true in the orthopneic patients. Consequently the latter usually had greater complementary air in the sitting posture and the former had more "room to breathe" in the recumbent position. These observations may throw some light on the facts that normal subjects may breathe slower when recumbent and that orthopneic individuals are likely to breathe slower when sitting.

#### *C The relations of the position of the head and of venous pressure to orthopnea*

Ernstene and Blumgart (1930) observed that in orthopneic patients elevation of the head without raising the body was often followed by distinct diminution in respiratory discomfort. They concluded that this relief was to be attributed to increased cerebral blood flow due in turn to diminished venous pressure. The data already presented (Table 1) indicate that the blood flow through the head is not changed by posture in patients with orthopnea. However, although we disagree with their conclusion, we have confirmed their observation (Table 4). In seven of eight observations on seven patients the ventilation was less with the head flexed than with it extended. As significant changes in respiratory rate and in vital capacity were not observed these data are omitted from the table. Six of the seven patients said that they were somewhat more comfortable with the head flexed although in most instances the difference was not striking.

In order to determine whether the increase in venous pressure produced by extending the head was in itself responsible for the increased respiratory discomfort felt by the patients further observations were

TABLE 4

Ventilation in recumbent patients with the head extended and with the head flexed

Subject	Chief diagnosis	Date	Degree of orthopnoea	Position of head	Ventilation per minute per square meter	Degree of subjective relief produced by raising head
D. W.	Hypertension	January 15	Necessity	Extended Flexed	6.05 6.56 5.94	Slight
A. B.	Hypertension	January 15	Choice	Extended Flexed	5.2 <sub>r</sub> 4.5 <sub>r</sub>	Slight
W. M. J.	Syphilitic aortic insufficiency	January 15	Necessity	Extended Flexed	4.62 3.82	Moderate
		January 17	Necessity	Extended Flexed	4.76 4.68	Slight
C. M.	Hypertension Emphysema	January 19	Necessity	Extended Flexed	5.12 4.76	Slight
J. L.	Asthma Emphysema Tuberculosis	January 14	Necessity	Extended Flexed	4.6 <sub>r</sub> 4.96	Slight
J. D.	Mitral regurg. Aortic insufficiency	January 15	Choice	Extended Flexed	6.63 5.99	None
W. C.	Hypertension	January 10	Choice	Extended Flexed	5.54 5.55	None

made on both normal and patients. A blood pressure cuff was put around the neck of the recumbent subject. The ventilation was measured for 3 minutes with no pressure, and successively after the cuff had been inflated to 15 mm. Hg with the patients and to 15, 25 and 35 mm. with the normals. The head was then flexed and the measurements at various pressures repeated. Finally the subjects were propped up in the sitting position and the same observations were made.

The results for the normals are shown in Figure 2 each point being the average value for four to six measurements. In each position increase in the pressure caused some increase in ventilation. At all de-

grees of pressure the ventilation was least in the horizontal position with the head flexed and most in the sitting position, the values for the reclining position with the head extended being intermediate. However, with moderate pressure (15 and 25 mm.) the increase over the

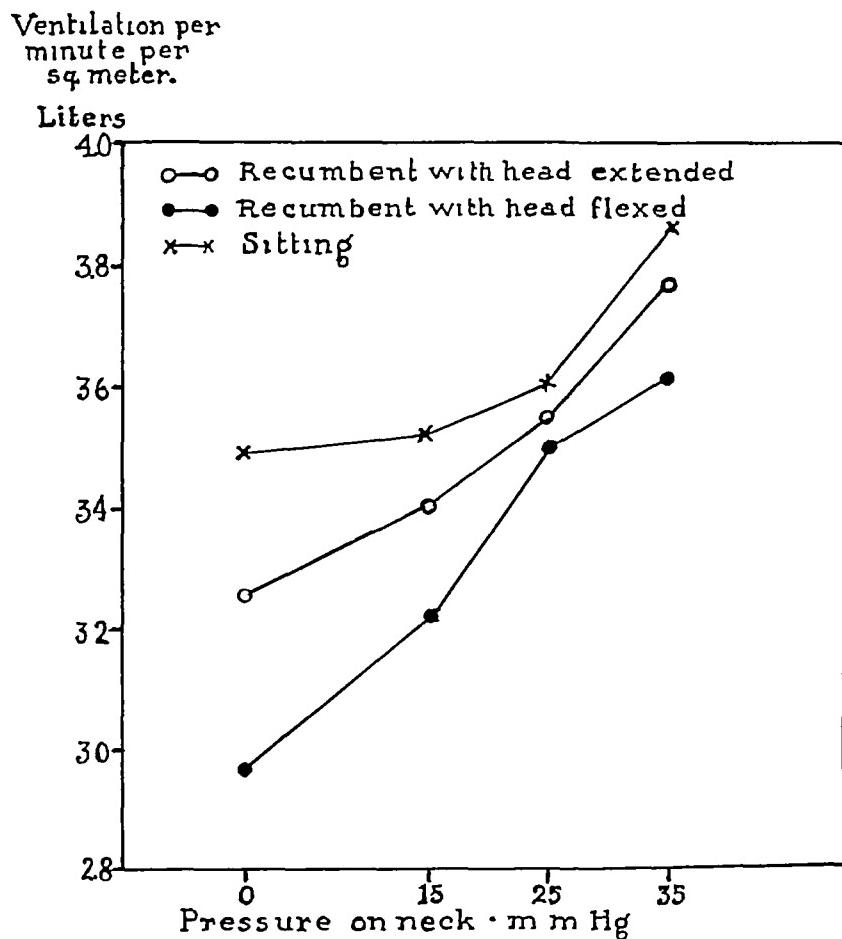


FIG 2

Shows relationship between pressure on neck and ventilation in various positions All subjects normal

value with no pressure was less in the sitting than in the two recumbent positions

The subjective sensations of the normal subjects are of especial interest In addition to the local sense of pressure on the neck which

was incidental to the procedure, all of them experienced an uncomfortable feeling of fullness in the head. The degree of this sensation was naturally proportional to the height of the pressure but each of the subjects noted that for any given pressure he was distinctly less uncomfortable with the head flexed than with the head extended and was least uncomfortable in the sitting position. *None of the subjects felt any shortness of breath.* If rise in venous pressure *per se* is an important cause of cardiac dyspnea they should have been quite short of breath because the venous pressures in the head were decidedly higher than those ordinarily found in patients with congestive heart failure.

Observations were made on the effect of artificially increasing the venous pressure to 15 mm Hg in five patients. Three of them had had congestive failure with orthopnea in the past but did not have orthopnea at the time the observations were made. In the recumbent posture their ventilation was measured with and without putting pressure on the neck. Significant increase in ventilation was not observed (Table 5). Subjective dyspnea did not occur. The other two patients had severe orthopnea and were studied in the upright position. Again significant changes in ventilation and in subjective respiratory distress did not take place when pressure was applied to the neck. These observations seem to be fairly conclusive proof that increase in venous pressure is not the major factor in the production of orthopnea.

These observations seem to us to clarify the significance of the position of the head and of venous pressure in the production of orthopnea. Regardless of the height of venous pressure recumbent individuals feel somewhat uncomfortable with the head extended (most people prefer to sleep on a pillow). The higher the venous pressure the greater is the discomfort in this position.

Distress of any kind tends to cause increase in breathing. This slight increase in ventilation causes no discomfort in normal subjects because of their large respiratory reserve (vital capacity). The orthopneic patient is already calling on his reserves and the slight increase in ventilation which comes when the head is extended causes further respiratory distress. It should be noted that the relative difference in ventilation on flexing the head was about the same (ten per cent) in the normal subjects and in the patients. However, the actual decrease in ventilation on flexing the head was almost twice as much in the patients.

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Ventilation per  
minute per  
sq meter.

Liters

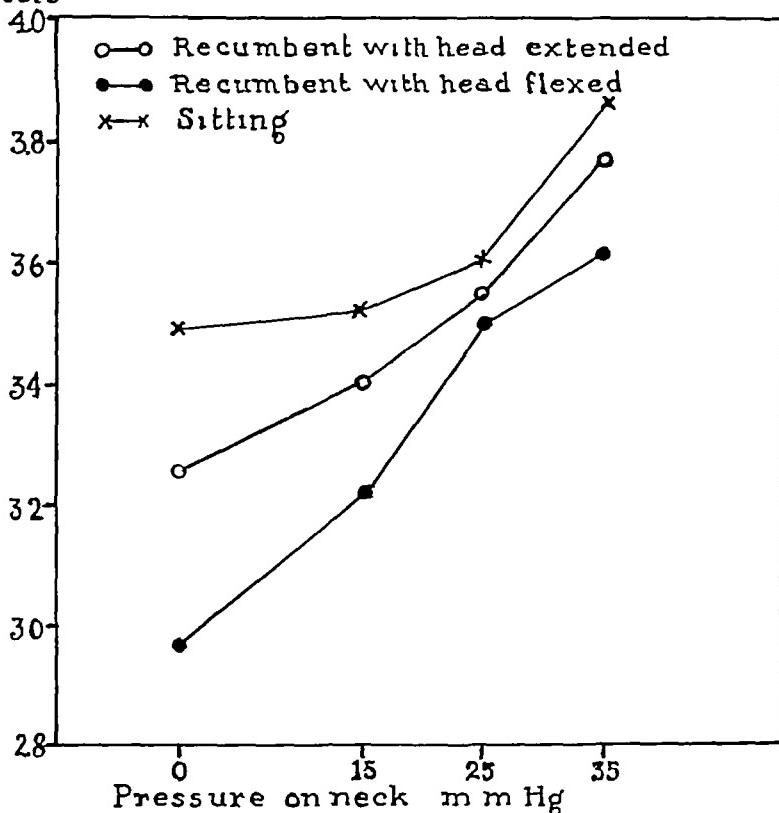


FIG 2

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## ORTHOPNEA

TABLE 5  
*The effect of pressure on the neck on ventilation*

Subject	Diagnosis	Degree of orthopnea	Position	Pressure on neck	Ventilation per minute per square meter	Degree of respiratory distress
F J	Bronchitis Cardiac hypertrophy	None	Recumbent	mm Hg 0 16	Liters 4.03 3.57	None
A C	Hypertension Cardiac hypertrophy	None	Recumbent	0 16	4.40 4.47	None
L C	Hypertension Cardiac hypertrophy	None	Recumbent	0 16	3.77 4.30	None
W C	Hypertension Cardiac hypertrophy	Necessity	Sitting	0 15	4.89 4.60	Slightly more dyspneic with pressure on neck
A L C	Syphilitic aortic insufficiency	Necessity	Sitting	0 15	7.26 7.73	Unchanged

It is possible that changes in venous pressure or in position of the head or neck may, through reflexes, have some influence on breathing. It is conceivable that alterations of the blood pressure in the carotid sinus is responsible, but of this we have no direct evidence.

## SUMMARY

Orthopnea is a complex phenomenon. A number of factors may play a rôle in its production. An attempt has been made to evaluate their relative importance in this study and the following conclusions have been reached:

1. *Decreased cerebral blood flow in the recumbent as compared to the sitting posture* apparently does not occur. The amount of oxygen taken out of the blood passing through the brain is, in orthopneic patients, almost exactly the same in the two positions and, although figures from different individuals show wide variations, the average figure is ap-

proximately the same as that found in normal subjects. Hence, changes in cerebral blood flow are probably of no significance in the production of cardiac dyspnea either in the sitting or recumbent postures.

*2 Oxygen saturation and acid base condition.* The arterial saturation often is somewhat greater in the sitting than in the horizontal position. The degree of change is frequently too small to be of great importance and even in recumbent orthopneic patients the arterial saturation is usually within normal limits. The carbon dioxide content of the arterial and internal jugular blood is usually normal and is relatively unaffected by posture. In both positions the carbon dioxide tension and hydrogen ion concentration of blood entering and leaving the brain are usually lower than the average normal and in most cases the values are not changed beyond the limits of error of the methods, by change of posture. In certain cases, particularly those with asthma, the pH may be low and the carbon dioxide tension high in the recumbent posture, and in such patients sitting up is followed by a shift toward alkalinity in these functions. Ordinarily such alterations in pH and CO<sub>2</sub> tension as are found in both positions are to be regarded as effects rather than causes of dyspnea. It is therefore evident that in the majority of patients with orthopnea due to congestive heart failure, deficient aeration of the blood in the recumbent posture is either of no significance or of only slight importance.

*3 The position of the head.* is of some significance in almost all orthopneic patients. The relief produced by flexing the head is not due to change in cerebral flow.

*4 Increased respiratory rate.* in the recumbent posture is also of importance in the production of severe orthopnea in certain patients.

The cause of the increased respiratory rate in patients with cardiac failure and the reason for the further increase, in some cases, on assuming the horizontal posture is not yet entirely known. It is not due to changes in the oxygenation or acid base condition of the blood.

*5 Diminution in vital capacity.* In patients with advanced cardiac failure a diminution is found invariably. In the sitting posture the patients respiratory reserve is much decreased, i.e., he is near the threshold of dyspnea. On lying down there follows a further decrease in vital capacity which is not only relatively but also usually actually

greater in patients with congestive failure than in normal individuals. In our patients the average increase in vital capacity in the sitting as compared to the horizontal position was 460 cubic centimeters or twenty-seven per cent of the recumbent value. The fraction of the vital capacity used per breath is therefore greater in the recumbent posture and this is, in large measure, responsible for their distress on lying down. Changes in vital capacity and lung volume are to be regarded as the most important causes of orthopnea.

These conclusions are in agreement with those of Peabody (1916-17) and his co-workers in indicating the importance of diminished vital capacity in cardiac dyspnea in general and with those of Christie and Beams, and of Field and Bock (1925) in regard to the significance of changes in vital capacity in the production of orthopnea.

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greater in patients with congestive failure than in normal individuals. In our patients the average increase in vital capacity in the sitting as compared to the horizontal position was 460 cubic centimeters or twenty-seven per cent of the recumbent value. The fraction of the vital capacity used per breath is therefore greater in the recumbent posture and this is, in large measure, responsible for their distress on lying down. Changes in vital capacity and lung volume are to be regarded as the most important causes of orthopnea.

These conclusions are in agreement with those of Peabody (1916-17) and his co-workers in indicating the importance of diminished vital capacity in cardiac dyspnea in general and with those of Christie and Beams, and of Field and Bock (1925) in regard to the significance of changes in vital capacity in the production of orthopnea.

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# A COMPARISON OF THE RACEMIZATION CURVES FOR URINARY, EDEMA FLUID, AND BLOOD PLASMA PROTEINS<sup>1</sup>

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## INTRODUCTION

The nature and origin of the urinary proteins in albuminuria has been a subject of considerable discussion. Presumably these proteins are blood plasma proteins which have passed through the capillary wall, however, there is some evidence that they may have originated from some other source.

The urinary proteins have been compared with other body proteins such as those from blood, liver and kidney by the precipitin reaction and by the effect of the protein upon smooth muscle from an animal which has been allergically sensitized. The biological reactions are very valuable for the identification of proteins but as was cited by Woodman (1) in the case of lactalbumin and serum albumin, the precipitin and anaphylaxis reactions may not always give the true picture. It is probably wise before drawing conclusions about the identity of a protein to compare the biological reactions with both physical and chemical means of identification.

There has been some study of the optical rotations for these proteins but the results of different workers have varied considerably due to the state of purity of the proteins used. Young (2) and Hardy and Gardiner (3) have shown that variations in the pH at which the readings are taken cause optical rotation for protein solutions to differ. In 1909 Kossel and Weiss (4) noticed that the optical rotation for protein solutions in dilute alkali at room temperature diminished for about two weeks and then became constant. This phenomenon was called race-

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<sup>1</sup> The contents of this paper were submitted by J W C as a partial fulfillment for the degree of Doctor of Philosophy



saturation. After being allowed to stand, preferably over night, the solution was filtered on a pleated filter and the precipitate washed with half saturated ammonium sulphate solution. The half saturated filtrate was then adjusted to the point of maximum precipitation for the albumin, by adding a little more ammonium sulphate and 10 per cent acetic acid. This point was at a pH of about 5.1. After allowing to stand for 24 hours the albumin was filtered out on a pleated filter.

From this stage the two fractions were subjected to the same treatment. They were dissolved in distilled water and diluted until the solution was less than one per cent.

The proteins were heat coagulated and the pH adjusted to the point of maximum precipitation by adding 10 per cent acetic acid. The solution was allowed to stand until the precipitate settled and the supernatant liquid siphoned off. Hot distilled water was then added until the original volume was reached and as soon as the precipitate settled this liquid was removed by siphoning. This process was repeated until no test could be obtained for sulphate, with barium chloride, or for ammonium with Nessler's reagent. This usually required 7 or 8 changes when large beakers were used. In the above procedure it was necessary to keep the supernatant liquid at the isoelectric point of the protein. As the ammonium sulphate was removed there was a tendency for the liquid in which the protein was suspended to become acid. To prevent this a burner was placed under the beaker containing the solution, each time when a new portion of hot water was added. If the solution was turbid a drop or two of dilute sodium hydroxide was added.

The protein was next transferred to a 250 cc centrifuge tube and centrifuged to remove as much water as possible. It was then treated, without removal from the tube, by boiling with 95 per cent alcohol three times absolute alcohol once, absolute alcohol ether mixture once and with ether twice, the liquid being centrifuged off each time, while it was still hot in the case of the alcohol. This gave a fine white powder which appeared to be lipid free.

#### METHOD OF RADICALIZATION

Woodman used one gram of protein for an analysis but in the present work the procedure was modified so only 0.2 gram of protein was used.

mization Certain of the amino acids obtained from these racemized proteins by acid hydrolysis were found to be optically inactive whereas if obtained before racemization they were optically active Dakin (5) ascribed this change to a keto-enol tautomerism of the  $=\text{CH}-\text{COH}$  groups in the protein molecule Thus amino acids with free COOH groups remain optically active during racemization while those in a peptide linkage become optically inactive By studying the optical rotations of the amino acids isolated from the protein before and after racemization he was able to learn something as to their position in the protein molecule as well as to the quantity of each acid present This is valuable as two proteins may contain identical quantities of the various amino acids and still be different This method was very long and tedious

Woodman (1) noticed that the optical rotation of the protein during racemization under constant conditions, if plotted against time, gave a definite curve He studied the racemization curves of a number of proteins in N/4 and N/2 alkali at 37° C and concluded that each protein has a characteristic racemization curve when obtained under the same conditions He also showed that heat coagulation or different means of isolating the protein did not effect the curve

It therefore appeared that to study the identity of the urinary, edema fluid and blood plasma proteins by this method would be of value

#### *Precipitation and purification of the proteins*

In this work the proteins were obtained from blood plasma, edema fluids and nephritic urines The pH at which the precipitation is carried out affects the fractionation of the proteins, therefore, the urine was adjusted to the same pH as that of the plasma and edema fluids, 7.4, before precipitation The blood plasma and edema fluids which were rich in protein were diluted 1:5 A volume of saturated ammonium sulphate equal to one half of that of the protein solution was added with stirring This produced one-third saturation which precipitated the fibrinogen and euglobulin The solution was allowed to stand a few hours and filtered In cases of nephritis this fraction was so small it was discarded

To the above filtrate, one-third of its volume of saturated ammonium sulphate solution was added, thus bringing it to one-half

Lactalbumin, which is probably closely related to serum albumin, gives a curve, which could not be mistaken for the serum albumin curve, as is shown by Woodman (1). At the beginning of the racemization period in N/4 alkali its optical rotation is 17° higher than that of serum albumin and at the end of the period it is still 3° high (In aqueous solution lactalbumin has less optical rotation than serum albumin) These two proteins have also been shown to be different by Hartley (6) and Crowther and Raistrick (7) using the Van Slyke nitrogen distribution method.

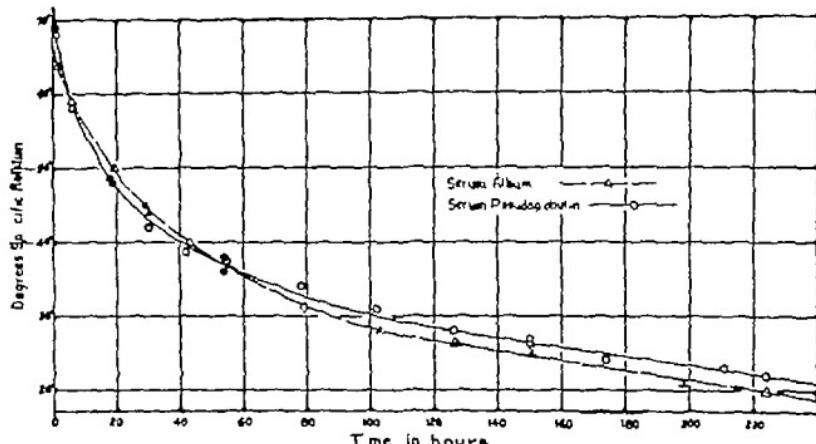


CHART 1 RACEMIZATION CURVES OF SERUM PSEUDOGLOBULIN AND SERUM ALBUMIN IN 0.5 N NaOH

With the technique used in this work if two proteins are identical the curves plotted from the data of either will be the same and no single reading should be over 2° from this curve. Very few readings will be over 1° from the curve.

#### EXPERIMENTAL

The proteins used in this experiment were obtained from the following cases:

*Case I* J. D., number C1128 age 19 diagnosed as chronic diffuse glomerular nephritis. A specimen of urine was obtained.

*Case II* F. H. number C3568 age 41 diagnosed as diffuse nephritis, diabetes mellitus and arteriosclerotic gangrene. A specimen of urine was obtained. The albumin globulin ratio of the blood was 1 : 1.

This did not affect the accuracy and the reading was simplified as the solution remained during racemization in the polariscope tube instead of a flask from which samples had to be taken for each reading. The protein, in a weighing bottle, was placed in a dessicator over calcium chloride for several days before the sample was weighed out. The 0.2 gram sample was placed in a 10 cc volumetric flask and 4 cc of carbon-dioxide-free water added. The flask was shaken until the protein was completely wet and dispersed into fine particles. While shaking the flask, 5 cc of normal carbon dioxide-free sodium hydroxide was added. The contents were diluted almost to volume and placed in an incubator at 38° C. Under these conditions solution was complete in 10 to 20 minutes and the volume was made up to 10 cc thus giving a 2 per cent protein solution in 0.5 normal alkali. Heat coagulated proteins from which the lipids had not been thoroughly extracted gave a turbid solution with alkali and could not be used. The solution was filtered into a 5 cm, jacketed T polariscope tube where it remained in an incubator throughout the racemization period. Optical rotation readings were taken every few hours at first and later only every 24 hours in a Schmidt and Haentsch polarimeter with an electric bulb and chromate filter (10 per cent potassium chromate solution, 30 mm thickness) as a source of light.

The experimental error in reading this type of polarimeter using 5 cm tubes with protein solutions is about 0.01° after one's eye has become accustomed to reading. An average of several readings gave the final result. However there are times when one's eye is less sensitive and a single reading may vary as much as 0.02°. In this work 0.01° reading on the polariscope was equivalent to 1° specific rotation.

In this method of identification of a protein one is not interested so much in a single reading as in the type of curve given by all the readings. There is only a few degrees difference in the specific optical rotations of serum albumin and serum globulin. The curves start at 67° and 72° respectively and at 6 hours the readings are both the same, 59°. However the pseudoglobulin continues to drop more rapidly, its reading remaining 2° or 3° below those of the albumin until 55 hours at which time the curves again cross each other, the albumin readings after this being less than those of the pseudoglobulin. The curves thus given, though never more than a few degrees apart, are easily distinguished (see Chart 1).

from the blood of V. P., a nephritic, gave the same curve (Chart 6) as that given by normal serum pseudoglobulin, thus indicating that the blood proteins of nephritics are normal.

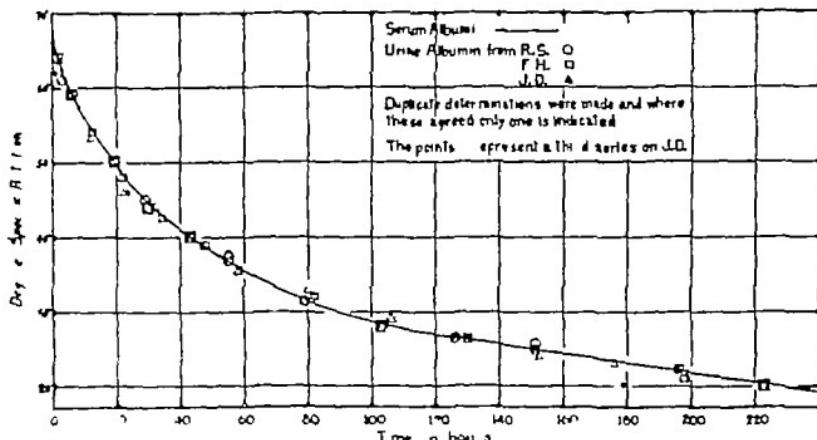


CHART 2 RACEMIZATION CURVES OF URINE ALBUMINS IN 0.5 N NaOH

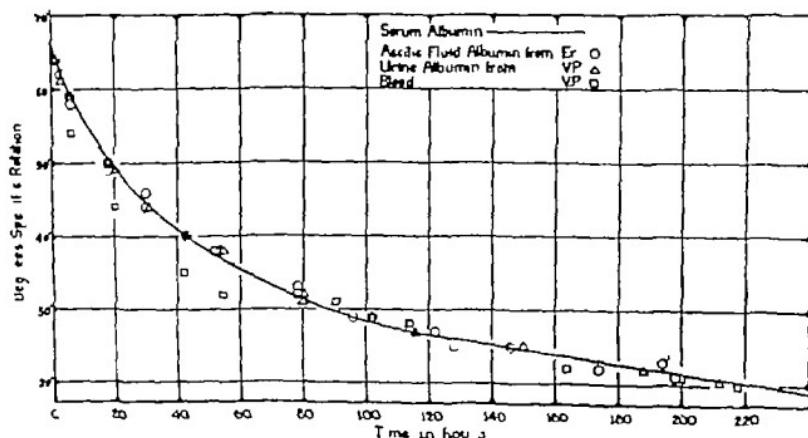


CHART 3 RACEMIZATION CURVES OF ALBUMINS IN 0.5 N NaOH

The pseudoglobulins obtained from the ascitic fluid of Er. (Chart 6) and from the chest fluid of Nu. (Chart 7) gave data for which the greatest variation from the curve for serum pseudoglobulin was two degrees, thus being within the limits of the experimental error present.

*Case III* R S, number D4616, age 32, diagnosed as subacute mixed nephritis Specimens of urine and chest fluid were obtained The blood protein ratio was 2.5 parts of globulin to 1 part albumin

*Case IV* E Nu, number C2521, age 32, diagnosed as tuberculosis of the lungs A specimen of chest fluid was obtained

*Case V* A Er, number E339, age 58, diagnosed as portal cirrhosis A specimen of ascitic fluid was obtained

*Case VI* V P, number A4452, age 15, diagnosed as acute nephritis Specimens of blood and urine were obtained Globulin was slightly in excess of the albumin in the blood proteins

The normal blood sample was obtained by pooling blood from four convalescent patients who had been in the hospital for peptic ulcer, heart failure, tuberculosis and a psychopathic condition respectively

#### *Comparison of the proteins by racemization*

Two series of readings were taken upon the racemization of both the serum pseudoglobulin and the serum albumin These are given in Chart 1 Racemizations for each protein were made in duplicate except for the chest fluid albumin of R S of which there was enough material for one determination only Three sets of readings were taken on the urine albumin of J D as the second set, due to an error, gave a curve about two degrees lower than the other two curves The nephritic blood from V P was not entirely lipid free This delayed the solution of the protein and the filtration so that the first reading which was not obtained until six hours later, was 5° lower than that of normal serum albumin The presence of lipids in proteins lowers the optical rotation This solution remained turbid throughout the period making reading difficult and inaccurate Therefore this data was disregarded in drawing conclusions

The racemization curves of the urine albumin from R S, F H, J D, and V P (Charts 2 and 3) are similar to the curves given by serum albumin The data obtained from the chest fluid albumin of R S and Nu (Chart 4) and from the ascitic fluid of Er (Chart 3) when plotted give curves similar to that of serum albumin It appears from these data that the albumins from these various sources are serum albumin

The pseudoglobulins obtained from the urine of the above patients, when racemized, gave data and curves (Charts 5 and 6) which are similar to those given by the serum pseudoglobulin Pseudoglobulin

from the blood of V. P., a nephritic, gave the same curve (Chart 6) as that given by normal serum pseudoglobulin, thus indicating that the blood proteins of nephritics are normal.

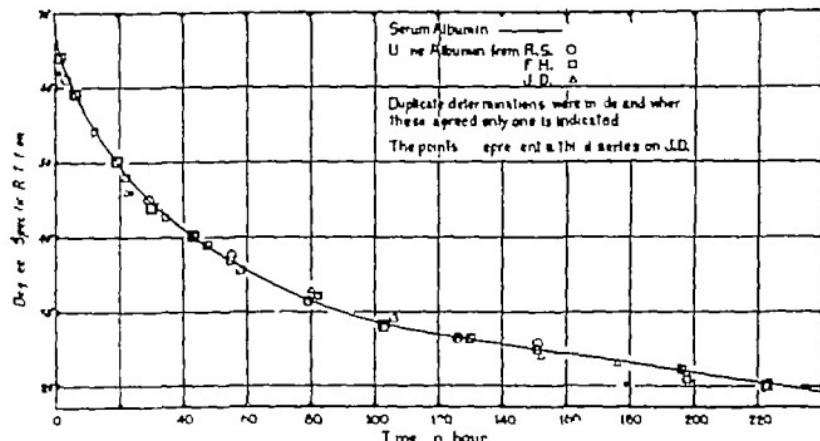


CHART 2 RACEMIZATION CURVES OF URINE ALBUMINS IN 0.5 N NaOH

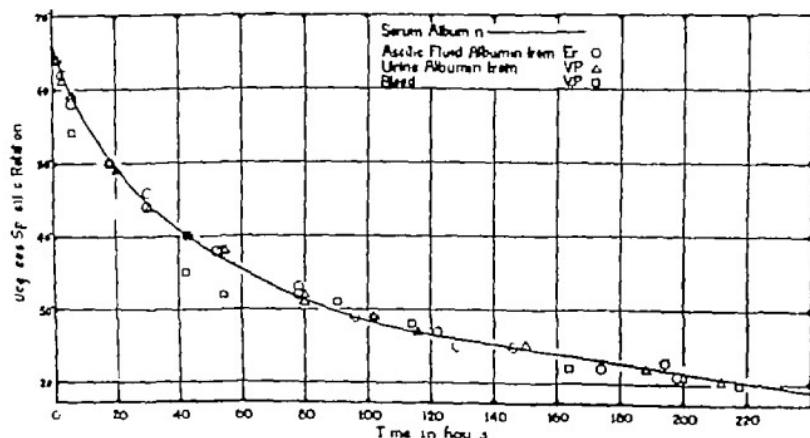


CHART 3 RACEMIZATION CURVES OF ALBUMINS IN 0.5 N NaOH

The pseudoglobulins obtained from the ascitic fluid of Er. (Chart 6) and from the chest fluid of Nu. (Chart 7) gave data for which the greatest variation from the curve for serum pseudoglobulin was two degrees, thus being within the limits of the experimental error present.

in reading the polariscope. However if one analyses these data closely it will be noted that the curves plotted from them are not exactly identical with that of serum pseudoglobulin. The curves given by these

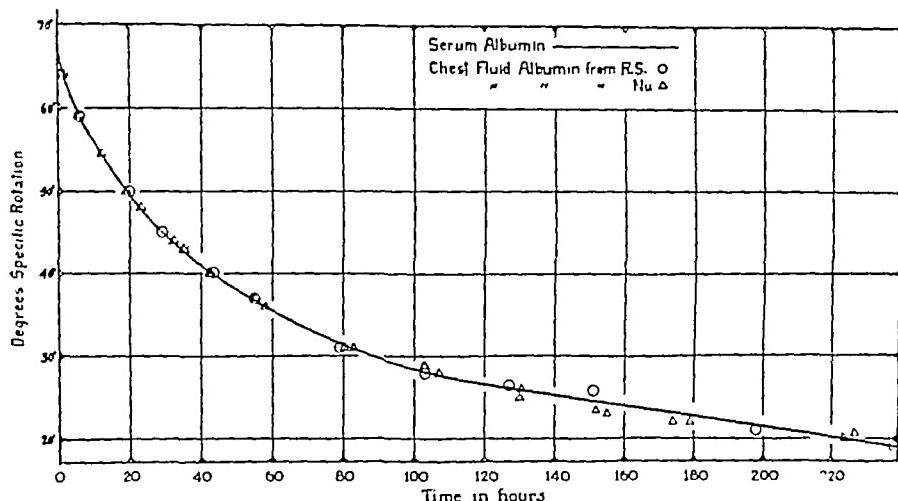


CHART 4 RACEMIZATION CURVES OF EDEMA FLUID ALBUMINS IN 0.5 N NaOH

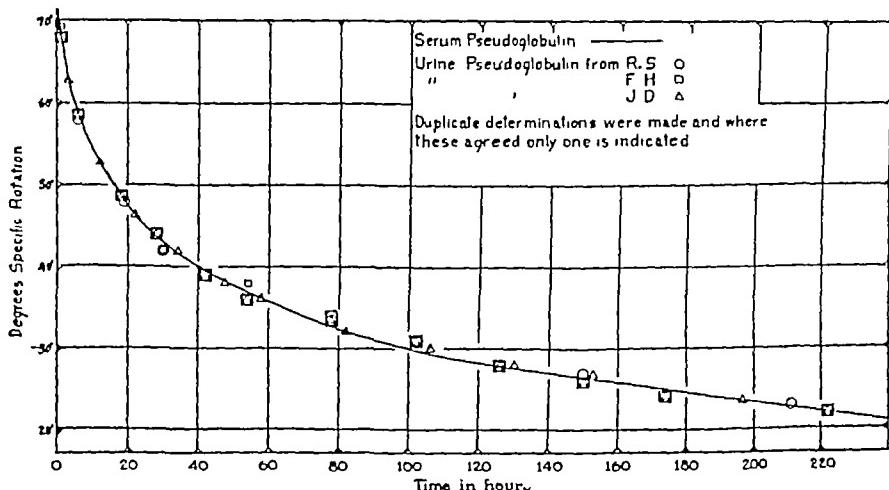


CHART 5 RACEMIZATION CURVES OF URINE PSEUDOGLOBULINS IN 0.5 N NaOH

two proteins are slightly above the normal curve at 25 to 45 hours and below it in the later part of the period. For this reason one might conclude that these proteins were not identical with the serum protein

However these curves suggest the possibility that serum albumin was present as an impurity and the authors feel that this was the case. Fractionation of proteins is carried out best when the solution contains

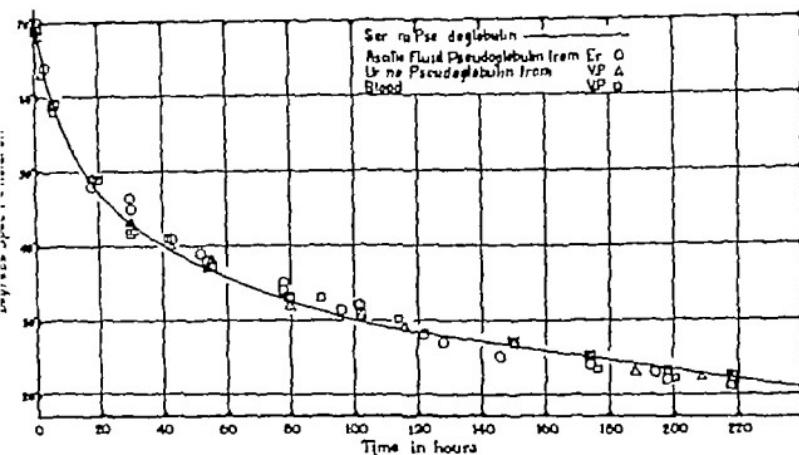


CHART 6 RACEMIZATION CURVES OF PSEUDOGLOBULINS IN 0.5 N NaOH

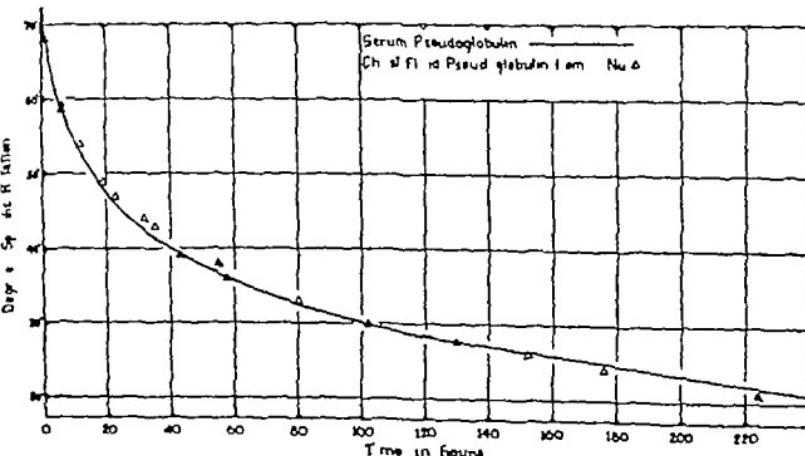


CHART 7 RACEMIZATION CURVE OF EDEMA FLUID PSEUDOGLOBULIN IN 0.5 N NaOH

about 1.5 per cent of protein. In these two instances, as was shown by the amount of protein obtained, the fluids were not diluted sufficiently before fractionation. Kauder (8) and Gibson and Banzhof (9) found

in reading the polariscope. However if one analyses these data closely it will be noted that the curves plotted from them are not exactly identical with that of serum pseudoglobulin. The curves given by these

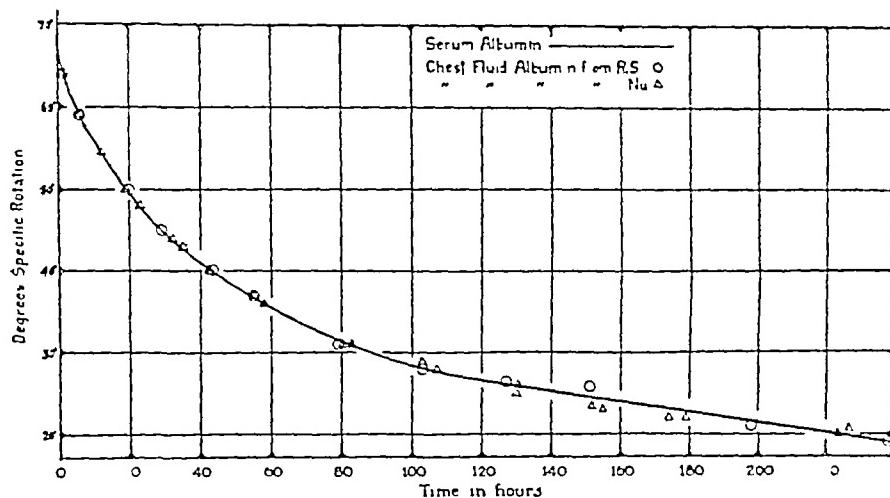


CHART 4 RACEMIZATION CURVES OF EDEMA FLUID ALBUMINS IN 0.5 N NaOH

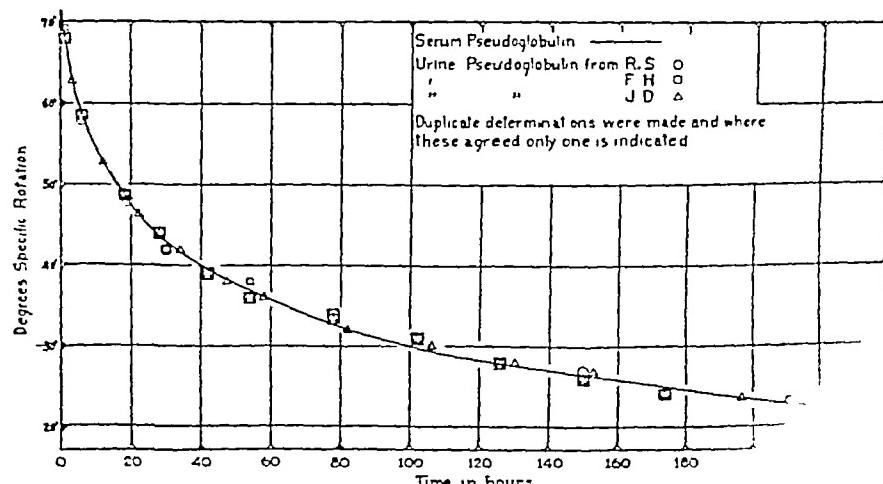


CHART 5 RACEMIZATION CURVES OF URINE PSEUDOGLOBULIN IN 0.5 N NaOH

two proteins are slightly above the normal curve below it in the later part of the period. For example, at 160 hours the serum pseudoglobulin is at 38°, while the urine pseudoglobulin is at 36°.

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# NUTRITIONAL EDEMA OBSERVATIONS ON THE RELATION OF THE SERUM PROTEINS TO THE OCCURRENCE OF EDEMA AND TO THE EFFECT OF CERTAIN INORGANIC SALTS

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(Received for publication May 23 1931)

The significance of the protein of the blood as a controlling factor in the distribution of fluids between plasma and tissues was first recognized by Starling (1) who postulated the existence of an equilibrium between capillary blood pressure, which tends to force blood fluids into the tissues and colloid osmotic pressure, which tends to draw fluid from the tissue spaces into the blood stream. Within the last few years the accumulation of experimental data has emphasized the importance of this equilibrium and produced evidence to support the view that a disturbance of this balance may be the direct cause of several different types of edema. In 1928 Leiter (2) reported experiments in which edema was produced in dogs by repeated blood-letting followed by centrifugation of the removed blood, separation of the plasma and reinjection of the corpuscles in Locke's solution. Similar experiments have been made by Barker and Kirk (3). It has also been shown by Denton and Kohman (4) and Maver (5) and Kohman (6) and confirmed by Frisch Mendel and Peters (7), that young rats fed on diets composed chiefly of carrots in many instances gradually develop edema. This diet is deficient in protein, but otherwise normal. In both types of experiments, i.e., plasmapheresis in dogs (2, 3) and low protein diets in rats (7), the appearance of edema has been regularly associated with a reduction of the blood protein. Barker and Kirk stated that the determining factor was the level of the serum albumin. In their dogs edema appeared when the albumin fraction had fallen to about 1 gram per 100 cc. and increased rapidly as the fall continued until, with a reading of 0.6 gram per 100 cc. ascites, pleural effusion,

edema of the legs, prepuce and scrotum were always found. Because the globulin fraction always increased as the albumin fell they found it impossible to foretell the time of appearance and amount of edema by the total protein level.

Investigations such as these have served temporarily to direct attention away from the kidney and toward the plasma protein as the factor most intimately associated with the causation of nephritic edema. Moore and Van Slyke (8) have found with nephritic patients that when the total protein of the blood falls below  $5.5 \pm 0.3$  gram per 100 cc., or that when the plasma albumin falls below  $2.5 \pm 0.2$  gram per 100 cc., edema is usually present. In a series of patients with nephrosis Barker and Kirk (3) observed the first appearance of edema when the serum albumin had been lowered to 1 gram per 100 cc. and stated that a decrease to 0.5 gram per 100 cc. was always associated with marked generalized anasarca, ascites and pleural effusion. Of even greater interest from the standpoint of the causes of edema is the group of patients who develop edema in the absence of any evidence of renal or cardiac disease. This type of edema has been known from ancient times, but was extremely prevalent just after the world war in Central Europe where the people were forced to subsist on vegetable diets for long periods of time. Maver (5) gives an interesting account of early references to this type of edema, an excellent review of observations made when it was prevalent in Europe has been published by Burger (9). The malady has been described under a variety of names—edema disease, nutritional edema, epidemic dropsy, camp edema, war edema, prison edema, etc. Schittenhelm and Schlecht (10) decided that rest and increased amounts of protein and fat in the diet were the important factors in relieving edema. From refractometer observations they calculated that the protein of the blood was usually reduced to between 4 and 6 grams per 100 cc. Jansen's (11) studies indicated that these patients were often in a condition of negative nitrogen balance. It seems highly probable that the edema which occurs in chronic wasting illnesses or as a symptom in other dietary diseases is regularly associated with low protein in the blood. Landis and Leopold (12), in a patient with inanition edema due to dietary restriction combined with tuberculous enteritis, found, at the first observation, a serum protein of only 3.6 grams per 100 cc. Wolferth (13) has given

the clinical records of two men with fecal fistulae who presented emaciation and edema in association with chronic alimentary disease. In these cases the proteins of the blood were 3.3 per cent and 4.6 per cent, respectively. Bruckman and Peters (14) reported that the edema of malnutrition appears to be referable to serum albumin deficiency, that it almost always develops when the serum albumin falls below 3 per cent and that it is seldom found when the albumin exceeds 4 per cent. Marriott (15) stated that the edema frequently present in celiac disease appears to be dependent upon a low concentration of protein in the blood plasma. One of us (16) has already given reasons for believing that the edema sometimes found in association with keratomalacia is not due to a lack of vitamin A. We hold an analogous belief as regards the edema of beriberi, a belief which finds support in an experiment of Harden and Zilva (17). They observed the development of edema in a monkey which was kept on a diet of 250 to 300 grams of polished rice daily in spite of the administration of enough yeast to furnish an adequate amount of vitamin B. In one patient with an edematous type of scurvy we have observed a reduction in the serum protein. Recently Peters and his co-workers (18) have been emphasizing the relation between malnutrition and serum albumin deficiency and have given evidence to support their belief that malnutrition or protein starvation is the direct cause of the protein deficiency. The association of low serum albumin with edema as a result of low protein in the diet has also been observed by one of us in cases of undernutrition (19).

The observations cited above all tend to emphasize the importance of a normal balance between capillary blood pressure and plasma colloidal osmotic pressure in maintaining the usual distribution of fluid between blood and tissues. It is evident, however, that other important factors must be concerned. Krogh (20) has stated that "the exudation and eventual reabsorption of fluid in the intercellular spaces will depend upon the capillary blood pressure, the permeability of the capillary wall, the efficiency of the lymph flow, and the metabolic activity of the tissue cells." Schade and Claussen (21) have added to these factors by pointing out the importance of mechanical pressure in the tissues. Certain tissues, as those under the eyes, because of their inherent softness, are particularly prone to edema even when not exposed to the aggravating effect of hydrostatic pressure. Moreover

after edema has developed, back-pressure from the distended parts tends to establish some degree of equilibrium so that unlimited passage of fluid into the tissues does not occur. Clinical evidence of the probable existence of still other factors of importance is not infrequently encountered. The aggravating effect of muscular exercise on the edema of malnutrition has been noted by Burger (9). The sudden occurrence of diuresis with fever in chronic tubular nephritis is familiar to many clinicians as also is the fact that other patients may exhibit steadily progressing edema in spite of the presence of fever. In other cases the reason for the sudden change may be utterly obscure. Linder, Lundsgaard and Van Slyke (22) have cited the case of a patient who, "after a prolonged acute nephritis with obstinate edema, began to excrete fluids with such rapidity that he lost in about ten days one-third of his body weight. There was no change in either plasma proteins or arterial blood pressure. The plasma proteins were extremely low and did not begin to regenerate until after the edema disappeared."

The effect of sodium chloride in aggravating the edema of nephritis is well known. Moore and Van Slyke (8) point to their experience with one patient in whom the plasma albumin was just below the critical level. Edema disappeared soon after admission, reappeared when salt was added to the diet and disappeared again as soon as salt was once more restricted. The effect on water retention of the administration of sodium bicarbonate has also occasioned much comment, particularly in patients with diabetes, in whom the combined therapeutic attempts to control sugar metabolism by dietary restriction and to combat acidosis by administering sodium bicarbonate frequently led to the development of edema. Because, in patients thus treated, edema did not invariably occur, Joslin and Goodall (23) concluded that some factor other than the sodium bicarbonate must be responsible for the edema. They did not attempt, however, to correlate their results with plasma protein determinations. That such a correlation would have aided in clarifying their results is suggested by the work of Peters, Bulger and Eisenman (24) who have shown that the plasma proteins are usually reduced in severe diabetes associated with chronic malnutrition, and the observation by Bruckman and Peters (14) of a definite relation between the edema of malnutrition and low serum albumin. An analogous increase in the edema of nephritis has been observed by

von Wyss (25) Falta and Quittner (26) have published the results of parallel observations on diabetic patients and on patients suffering from war edema. In both instances the combined administration of sodium chloride and sodium bicarbonate led to a striking aggravation of the edematous state. In addition to the effect just mentioned other observers have shown that the action of potassium salts may be the reverse of that of sodium salts and lead to elimination rather than accumulation of edematous fluid. With edema in diabetes this has been reported by Falta (27) and Boenheim (28) and with edema in nephritis by Blum (29) and by Kempmann and Menschel (30). In addition to studies of sodium and potassium much has been written of the specific action of the bivalent calcium ion in various types of edema. To review these studies would lead us astray, but we may note that Maase and Zondek (31) as well as E Schultz (32) observed that acute war edema was influenced favorably by the administration of this salt.

#### *Observations on serum proteins in nutritional edema*

In China an unusual opportunity has existed for observing patients with edema primarily of dietary origin. Dietaries published by H Wu and D Y Wu (33) have shown that the bulk of protein and energy in the diet of the peasant and labouring classes in China is of vegetable origin being furnished chiefly by the cereal grains wheat, rice, corn, and millet. Such a diet contains less than the optimum amount of protein, relatively small amounts of fat, calcium and phosphorus, and is often deficient in vitamins A and D, and sometimes in vitamin B. Edema of the type under discussion occurs sometimes alone, sometimes in association with other deficiency diseases and often as a symptom in other illnesses particularly chronic dysentery. In common with other observers we have found the blood proteins regularly reduced in such conditions. In Chart I are shown the results of fifty four determinations of the blood proteins<sup>1</sup> in eighteen patients who at the time of hospital admission exhibited edema without evidences of cardiac or renal disease. Most of them were admitted with other complaints, but in all we felt that a restricted diet was the chief cause of the edema. A number suffered from chronic dysentery, several from various forms of

<sup>1</sup> The serum proteins were determined by Howe's method (*J Biol Chem*, 1921, *xlix*, 109)

tuberculosis, and in several the edema was associated with other deficiency diseases (rickets, tetany, keratomalacia, and scurvy). Five patients died before the edema disappeared, four could not be followed for various reasons, but on the remaining nine a total of twenty-one examinations of the blood serum for protein content were made after

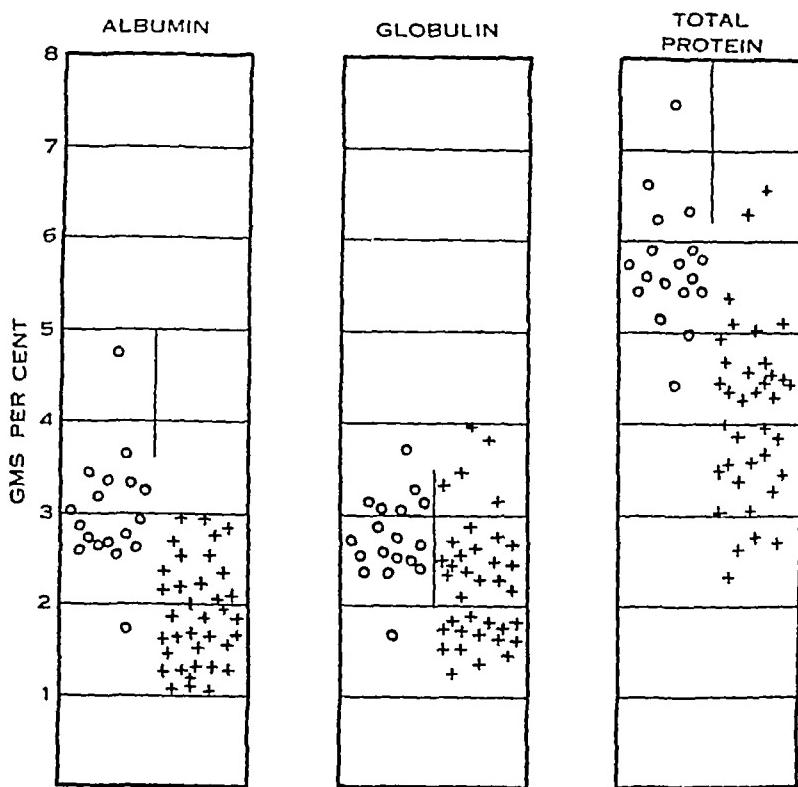


CHART I SUMMARY OF 54 OBSERVATIONS OF THE SERUM PROTEINS OF 18 PATIENTS WITH NUTRITIONAL EDEMA

+ indicates estimations made during the stage of edema, o indicates estimations made after the disappearance of edema, vertical lines in the middle of each column indicate the range of normal variation as given by Moore and Van Slyke

subsidence of the edema. These data have been arranged graphically in Chart I to demonstrate the relation between the serum protein level and the presence or absence of edema. They may be compared with figures for normal subjects collected by Moore and Van Slyke (8), which are 6.2 to 8.0 grams per 100 cc for total plasma protein, 3.6 to

5.0 grams per 100 cc. for albumin, and 2.0 to 3.5 grams per 100 cc. for globulin. It will be seen that edema was never observed when the serum albumin was greater than 2.9 grams per 100 cc. and, with one exception, was never absent when the albumin was less than 2.5 grams per 100 cc. The single patient who maintained a low serum albumin of 1.73 grams per 100 cc. after edema had disappeared had a globulin of 3.71 grams per 100 cc., the total serum protein was, therefore, 5.44 grams per 100 cc. The possibility of analytical error is not excluded. The figures for total protein can also be correlated rather closely with the presence and absence of edema, the critical level being close to 5.0 grams per 100 cc. There are several exceptions on either side of this critical level. The two instances in which a total serum protein greater than 6.0 grams per 100 cc. was associated with edema both occurred in the same patient during a period of slightly more than a month when edema was receding but had not entirely disappeared. The albumin values corresponding to these high total protein figures were 2.33 and 2.75 grams per 100 cc. and thus were within the zone of possible edema. On the other hand, it is seen that normal or even increased globulin values frequently occurred in association with edema and one must conclude that this fraction plays at least only a minor rôle in preventing the accumulation of interstitial fluid. Nevertheless, subnormal globulin values, that is, values below 2.0 grams per 100 cc., generally occurred only in edematous patients. It is probable that the protein-deficient diet tended to reduce both albumin and globulin fractions, the former more rapidly than the latter, so that by the time deficient globulin was observable it was always associated with deficient albumin. Lowered serum globulin may thus be regarded as an indicator of concomitant albumin deficiency and not as a direct cause of edema. The extreme variability of the globulin fraction renders reversal of the albumin-globulin ratio a much less constant feature than in nephrosis patients. During recovery both protein fractions increase. In the initial stage while there is still a distinct albumin deficiency, globulin rises rapidly, sometimes above the normal limits. As the process of recovery goes on the albumin continues to rise more slowly, while the globulin either sinks or remains constant until the normal ratio is re-established. The initial changes take place rather rapidly. Final adjustment may not occur for several months.

*Observations on salt metabolism*

With most of the patients of this series the associated illnesses for which hospital care was solicited were too severe to permit extended metabolism observations. To have withheld adequate nourishment even temporarily might seriously have affected the course of their diseases. With two patients, however, we felt justified in offering for a while a diet which was essentially the same as that which they had been eating for many months previously. With them we were able to confirm the observations of Falta and Quittner (26) who studied patients with war edema and to correlate this effect with the level of serum proteins.

*Case 1 Y H H, hospital number 27785* The patient, a boy aged 11 years, was admitted on March 28, 1930. He had recently been brought from one of the famine districts of China to Peiping and placed in an orphanage. A satisfactory past history could not be elicited except that his condition had failed to improve after arrival in the city. The lower extremities were swollen and deeply pitting edema could be demonstrated over the dorsa of the feet and up the legs as far as the knees. The upper portion of the body was in striking contrast to the lower, where edema masked the true state of nutrition. The ribs were outlined on the thoracic wall, and the muscles as well as the subcutaneous tissue about the shoulders and upper extremities appeared atrophied. Examination of the heart revealed only normal findings and repeated urine examinations failed to disclose any evidence of renal disease. The tendon reflexes were normally elicited. At the time of admission and periodically throughout his stay in the hospital the patient had mild diarrhea. *B. dysenteriae* were recovered from the stools which, however, were never clinically dysenteric in character. Throughout the period of study the patient had fever, the temperature course being hectic and varying between slightly subnormal values and 38° C. At first we were in doubt as to the cause of this fever but later unmistakable evidence of tuberculosis of the peritoneum appeared.

The record of our observations is shown in Chart II. The diet at the time of admission was composed of millet, corn, rice, and small amounts of green vegetables. No attempt was made to make it an adequate diet from the vitamin standpoint. We endeavored merely to continue the foods to which the patient had been accustomed. The diet fur-

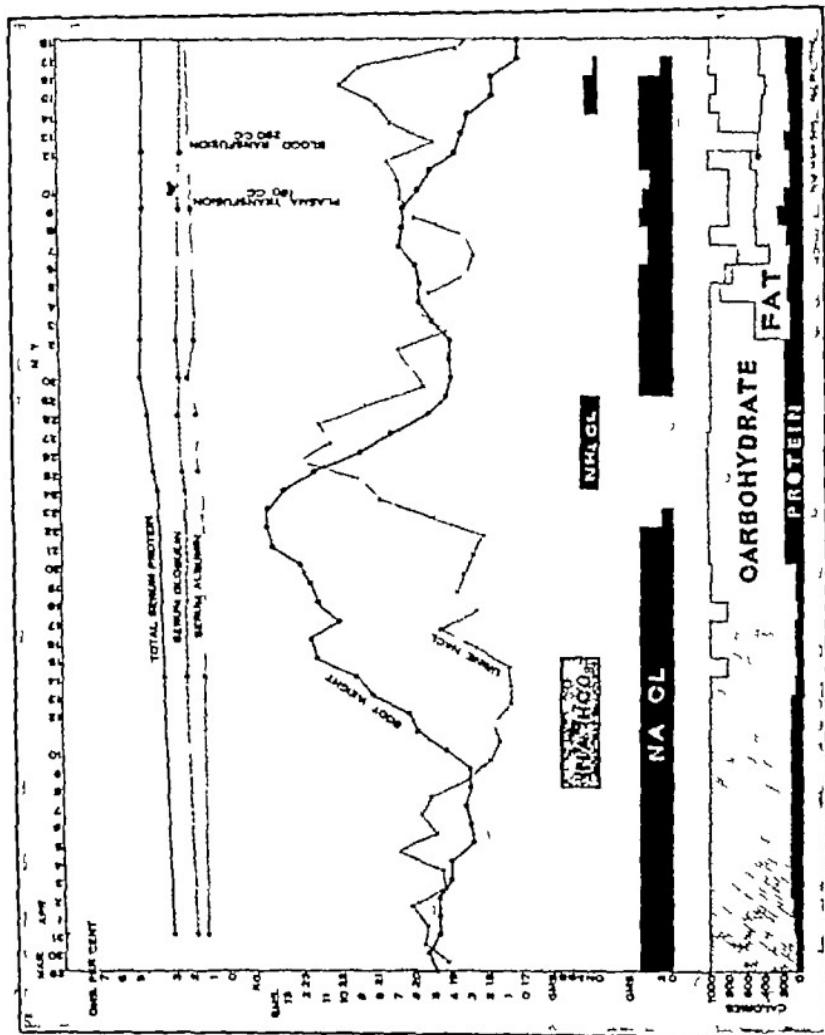


CHART II CASE I METABOLISM OBSERVATIONS

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been so charted. Due to refusals, however, which were not accurately recorded, the actual intake was slightly less than this and calculated roughly from the known daily refusals of protein, can be estimated at 52 grams per day. During this period the excretion of sodium chloride varied between 4.12 and 6.82 grams per day and averaged 5.23 grams. The patient was, therefore, certainly not retaining sodium chloride, a conclusion which is corroborated by a slight steady decline in body weight from 19.4 to 18.5 kgm. On April 8 at the termination of the ten day initial period the administration of 7.2 grams of sodium bicarbonate in addition to the 6 grams of sodium chloride was commenced.<sup>3</sup> Twenty four hours later the patient weighed 18.5 kgm, the same as on the previous morning. However during the days that followed until the morning of April 15 when the sodium bicarbonate was discontinued the patient's weight increased rapidly, reaching 22.6 kgm at the end of the period. The total gain during these seven days was therefore, 4.1 kgm or more than one-fifth of the initial body weight. Edema had increased tremendously. The scrotum was markedly swollen pitting to a depth of 3 cm could be produced over the sacrum the face was much puffed and the eyes nearly closed. Concomitantly with the increase in weight and edema the excretion of sodium chloride in the urine steadily decreased and during the last three days of the period was less than 1 gram per day. The average daily chloride excretion during the seven days of sodium bicarbonate administration was 1.55 grams as contrasted with 5.23 grams during the previous period. The decrease in chloride excretion was accompanied by depression of the volume of urine voided. The depression became more marked each day and the output reached a low figure of 280 cc. on the last day of the period. The average volume of urine during the period was 1009 cc. daily as contrasted with 1477 cc. during the previous ten days. This decrease occurred in spite of a voluntarily increased fluid intake, which averaged 1706 cc and 2043 cc during the two respective periods.

<sup>3</sup> When the factor of body weight is taken into account this dosage is seen to be close to the lower limit of that used by other investigators who studied adults. Thus Kempmann and Menschel (30) gave approximately 23 grams per day Boenheim (28) 25 grams per day and Falta (27) as much as 50 grams per day to a patient whose weight was varying between 52 and 63 kgm.

After the administration of sodium bicarbonate was stopped, sodium chloride was continued as before until after the morning dose on April 22. The excretion of chloride in the urine now increased, averaging 2.99 grams of sodium chloride daily, but did not return to the earlier level of 5.23 grams. The retention of salt thus indicated was corroborated by a continued though slower gain in weight, the patient weighing 24.0 kgm on the morning of April 22. On April 20 dried skimmed milk was added to the diet so as to bring the daily protein intake up to 50 grams but as the effect of this addition was not immediate and as the patient was exceedingly uncomfortable we did not feel justified in continuing the sodium chloride for a longer time. The effect of omitting it was immediate. On April 22 only the morning dose of 2 grams of sodium chloride was given but during the day the patient excreted 4.95 grams of sodium chloride. From April 24 to 28 the patient was given 4 grams of ammonium chloride daily. This salt had no effect on the rising excretion of chloride which continued until a maximum of 12.01 grams was reached on April 25. During the entire period of ammonium chloride administration the chloride excretion was from 2 to 2.75 times greater than the intake.<sup>4</sup> As is shown graphically in the chart the rising chloride excretion of this period was accompanied by a precipitate decline in body weight which fell from 24.0 kgm on April 22 to 19.1 kgm on April 29. The degree of edema was correspondingly lessened.

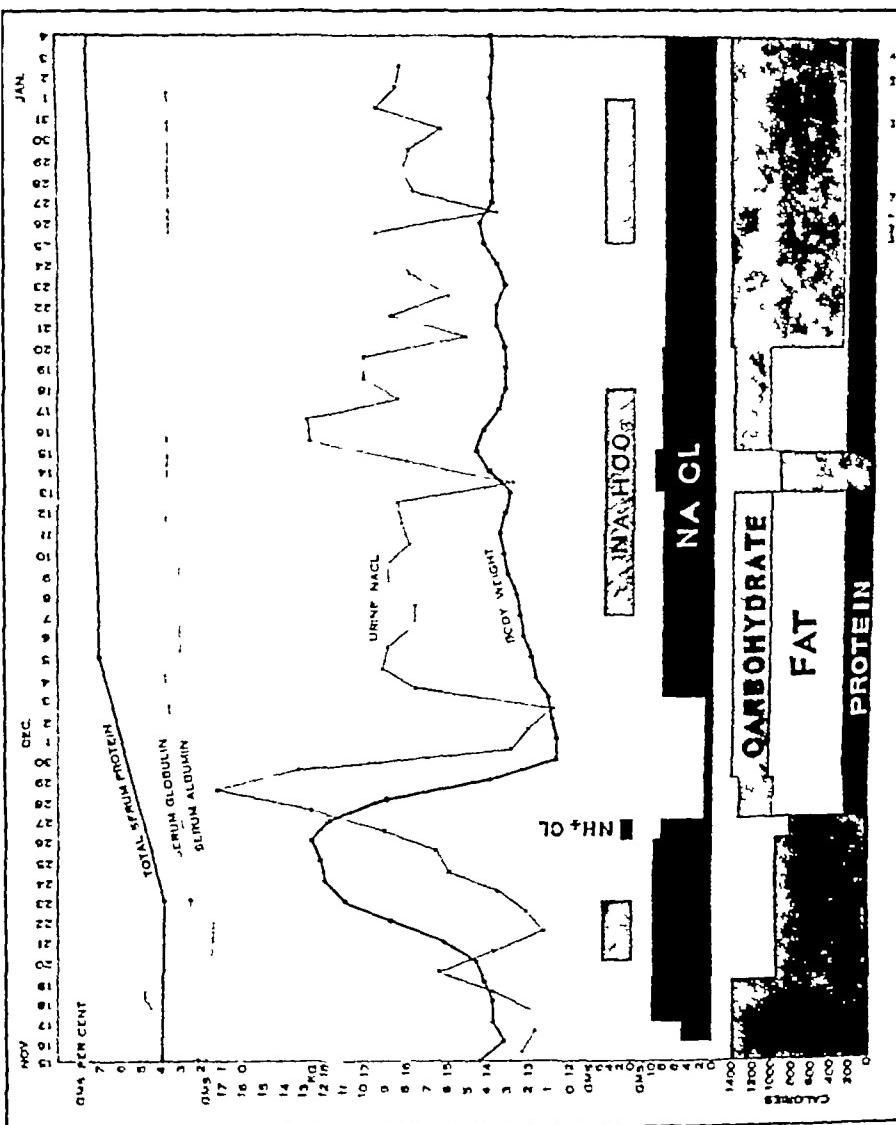
From this time on our observations were directed toward ascertaining whether in the presence of a normal intake of sodium chloride the remaining edema could be removed by other means. The addition of fat to the diet on May 2 produced no significant change. Plasma transfusion on May 9 and blood transfusion on May 12 produced no measurable change in the level of blood protein. These treatments

<sup>4</sup> As we shall see, when the next case is considered, it is probable that the ammonium chloride had no significant effect which would not have been observed if had been omitted. It was given in an attempt to render the patient more comfortable as quickly as possible, the rational being its known diuretic action and the possibility that an acid-producing salt might oppose the effect of the previously administered base-producing sodium bicarbonate. At all events the result strongly suggests that chloride given in combination with the ammonium radicle was much more easily excreted than when combined with sodium.

were, however, followed by a gradual decline in weight, though not to the point of the disappearance of edema. Inasmuch as the second transfusion was followed by a severe febrile reaction this form of therapy was not continued. From April 29 to May 15 the chloride excretion varied considerably from day to day, but averaged 5.81 grams daily as contrasted with an average intake of 6.06 grams (including chloride given as ammonium chloride).

*Case 2 L P C, hospital number 26208.* The patient, a boy aged 9 years, was admitted on November 14, 1929. Like the preceding patient he came to us from one of the city orphanages. The diet had been similarly restricted and he spoke feelingly of the inadequate quantities served. On admission he weighed 14.3 kgm and had edema which in degree and distribution was similar to that shown by the previous patient. Examination of the heart revealed only normal findings. The urine at no time showed albumin and the nonprotein nitrogen of the blood was 22 mgm per 100 cc. The tendon reflexes were all easily elicited. The composition of the diet during his stay in the hospital is indicated in Chart III. From November 14 until November 27 he was given a porridge made from millet and rice with added sugar. From November 27 until December 13 he was allowed a general diet containing liberal quantities of milk and egg. This diet furnished 71 grams of protein per day. On December 13 and 14 an attempt was made to return to the diet of admission but the patient complained so constantly and so persistently that it could not be continued. From December 20 until the end of the period of observation a liberal allowance of protein (egg albumin and dried skimmed milk) was continued, but the fat was reduced as much as possible and the bulk of the energy requirement supplied with carbohydrate.

The total serum protein at the time of admission was 3.98 grams per 100 cc., the partition being albumin 1.84 grams and globulin 2.14 grams. On November 23 after some days on the protein inadequate diet, the total serum protein was 3.83 grams per 100 cc., with 1.29 grams of albumin and 2.54 grams of globulin. Following an adequate diet the return of the blood protein to a normal level was extraordinarily rapid. An analysis done on December 5, the ninth day of this period, showed a total serum protein of 6.93 grams per 100 cc. of which 3.08 grams were albumin and 3.85 grams were globulin. As the figure



### CHART III CASE 2 VIETNAMESE OBSERVATIONS

for total protein was now normal further estimations were not made until a final analysis on February 14 showed a total serum protein of 7.33 grams per 100 cc., the partition being albumin 3.71 grams and globulin 3.62 grams.

With this patient, then it was possible to observe the response to the administration of sodium bicarbonate and sodium chloride both during the period of deficient plasma protein and again after the protein had been restored to normal. The response while the protein was still low was quite analogous to that observed with the previous patient. The combined administration of these salts produced an immediate fall in the chloride excretion and a rapid increase in body weight and in degree of edema. This accumulation of edema fluid in the tissues did not stop with the discontinuance of bicarbonate, although the daily weight increments were considerably lessened and a daily increasing excretion of chloride indicated gradual readjustment of the salt metabolism. A single dose of 2 grams of ammonium chloride given on November 26 could have had no significant effect. On the morning of November 27, in order to relieve the patient's extreme discomfort as rapidly as possible, he was given a liberal diet from which all added sodium chloride was omitted. The narrow black band on the chart representing a daily intake of 1.45 grams sodium chloride during the period from November 27 to December 2 inclusive indicates the chloride naturally present in the diet without the addition of salt. These changes were followed by immediate diuresis, marked increase in the excretion of sodium chloride, rapid decline in body weight and disappearance of edema. As with the previous patient, the greatest excretion of chloride occurred after chloride had been removed from the diet.

On the morning of December 3 sodium chloride, 7.5 grams daily, was again added to the diet. The chloride excretion immediately rose to correspond with this intake. As previously stated, an analysis of the blood on December 5 revealed that the total serum protein had already returned to normal and the albumin fraction had risen to 3.08 grams per 100 cc. The patient had commenced to gain weight slowly without the reappearance of edema. On December 7 the daily administration of 4.8 grams sodium bicarbonate was again started. This time there was no response by the production of edema, there was no increase in the daily increments of gain in weight and the chloride excretion was not

depressed. On December 13 with the thought that the high fat and relatively low carbohydrate provided by the diet might conceivably be a significant factor, the patient was returned to the diet of admission. Unfortunately he could be persuaded to eat this diet for two days only and this phase of the experiment could not be continued. The first day on this diet produced a sudden drop in the chloride excretion to 2.99 grams, but during the second day the previous excretion of slightly more than 8 grams was reattained. The following two days after return to the high fat diet disclosed a negative chloride balance in compensation for the short period of retention.<sup>5</sup>

On December 18 the administration of sodium bicarbonate was discontinued and on December 20 the diet modified so that, although it still furnished adequate protein, the fat was reduced as low as possible (3.75 grams per day) and the bulk of the energy supplied by carbohydrate (283 grams per day). This diet was taken willingly by the patient. Sodium bicarbonate was again started on December 25 and continued through December 31. No significant gain in weight occurred and edema did not develop. During the five days on this diet before bicarbonate was given the chloride output averaged 7.38 grams daily during the seven days of bicarbonate administration it averaged 7.64 grams.

#### COMMENT

In the present stage of our knowledge it would be unwise to attempt an exact explanation of the factors involved in disturbing the water balance in these patients. The relation between plasma protein and fluid distribution in the body has been discussed already, but the problem presented by the alteration in response to inorganic salts would seem to be more complicated. To conclude that the combination of sodium chloride and sodium bicarbonate is more able to cause water retention than sodium chloride alone is not justified, although we are not sure that this is not the case. It is quite possible that, if we

<sup>5</sup> We are unable to explain this temporary depression of the chloride excretion occasioned by the dietary shift but may note that such temporary depressions are not infrequent when a diet high in carbohydrate is substituted for one high in fat. With our patient it would appear that, after a temporary imbalance, the salt metabolism was able to readjust itself and would have remained normal subsequently even if it had been possible to continue the diet. Such an assumption finds support in the subsequent observations.

had increased the dose of sodium chloride so much that the total sodium intake equalled that of the two salts together, the effect would have been the same. However, such a large proportion of sodium chloride would have rendered the diet not palatable and it proved more practicable to utilize the mixture. The trend of present day opinion as expressed in the literature is to regard the cation sodium as a more important factor in the causation of edema than the anion chloride. Our data accord with this point of view. It is conceivable that an increase in the intake of sodium beyond the amount which can be excreted leads to retention, which in turn, and in order that the normal composition of the body fluid may be maintained, is accompanied by retention of both chloride and water. Moreover, whatever the mechanism which produces this effect, it would appear to bear a close relation to the serum protein level. It is, therefore, tentatively suggested that the threshold for the excretion of sodium may be controlled by the level of serum protein. Use of the word "renal threshold" is purposely avoided as there is no evidence to support the view that damaged renal function is concerned with the effects observed. We are also aware that the impoverished nutritional state which seems always to accompany marked depletion of the serum protein may in itself be concerned with the alterations observable in the water balance. With our second patient however, in whom the administration of an adequate diet produced prompt regeneration of the serum proteins, but only relatively slow improvement in nutrition, an intermediate stage existed when the serum proteins were normal and malnutrition was still extreme. The fact that during this period the characteristic salt effect was not observed, argues against a direct relation between malnutrition and the salt effect and suggests a closer relation to the level of serum protein.

#### SUMMARY

1 The relation of serum protein deficiency to various types of edema, both experimental and clinical, is discussed. Attention is also called to other factors and in particular to the rôle of several inorganic salts in modifying the effect of protein deficiency alone.

2 In a series of patients suffering from nutritional edema, who were studied both during the active and convalescent stages, it was found that when the level of serum albumin was greater than 2.9 grams per

100 cc edema was never observed and that when the level fell below 25 grams per 100 cc edema was invariably present. The correlation with total serum protein although not so close was sufficient to indicate a critical level for edema close to 50 grams per 100 cc. Serum globulin was exceedingly variable decreased, normal and increased values all were found in association with edema. Decreased globulin generally occurred only when the albumin was also decreased and thus was usually present in association with edema. No relation of cause and effect was considered, however, to exist between lowered globulin and edema.

3 The results of metabolism observations on two of these patients are given. In both cases the combined administration of sodium chloride and sodium bicarbonate led to rapid gain in weight, marked increase in edema and depression of chloride excretion in the urine. In one of the patients these effects could not be reproduced after feeding of an adequate diet had resulted in a return to normal of the serum protein.

4 The impossibility of offering an exact explanation of all the effects observed is pointed out, although it is stated that the results are in harmony with the view that the cation sodium is more intimately related to the causation of edema than the anion chloride.

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135 Study of Dietaries in Peking

## STUDIES ON THE ELECTRICAL SYSTOLE ("Q-T" INTERVAL) OF THE HEART

### II ITS DURATION IN CARDIAC FAILURE

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The clinical recognition of heart failure rests chiefly upon the symptoms produced by inadequate circulation resulting from myocardial exhaustion. Organic lesions, such as defects of valves, damage to the conducting system, and generalized arteriosclerosis, are no longer looked upon as signs of cardiac insufficiency, although they undoubtedly handicap the heart in performing its function. With the exception of auricular fibrillation and heart block as shown in objective records there is no physical or instrumental sign that is pathognomonic of heart failure. Moreover the physical signs which taken together with the history lead to this diagnosis must be considered to represent a relatively late stage in the process. From the point of view of treatment it is particularly desirable to detect the early stages. An attempt was therefore made to seek direct evidence of cardiac failure in demonstrable changes in cardiac dynamics. Considering the means at hand it was decided to investigate the "Q-T" interval of the electrocardiogram in patients presenting the clinical symptoms and signs of myocardial insufficiency.

The question of the significance of the "Q-T" interval will be briefly discussed later. In any case it is clear that the phenomenon may possess a pathological interest of its own, independent of other considerations.

The measurements were made under the conditions described in the previous paper (1), except that for patients with severe heart failure records were made with the patient sitting or half reclining. No records were taken soon after exertion or exercise and patients were al-

were kept at rest in the sitting or lying position for at least five minutes before the record was made. No patient known or suspected to have taken digitalis or any other drug having a known cardiac action is dealt with in this study.

The results of a preliminary investigation of individuals without heart failure have been previously published (1). A high degree of correlation between "Q-T" interval and "R-R" interval was found, as might be expected (see Table 4). The simple formula " $Q-T = K\sqrt{R-R}$ " has been used, in which the "Q-T" and "R-R" intervals are expressed in seconds and "K" is a constant. This formula expresses fairly well the normal relationship for ordinary cardiac rates. It has, however, many limitations and is used only for its convenience. The relations of the results do not depend upon its exact form.

The arithmetical mean is calculated in the usual way, the standard deviation by the formula,  $SD = \sqrt{\frac{\sum d^2}{N}}$ . The formula used for the standard error is,  $SE = \frac{SD}{\sqrt{N}}$ . The error of differences is  $E_d = \sqrt{E_1^2 + E_2^2}$ . The correlation coefficient is calculated by the formula  $\frac{\Sigma(1y)}{N(\sigma_1)(\sigma_2)}$  and its probable error by  $0.6745 \frac{1 - r^2}{\sqrt{N}}$ .

The normal results are best stated and dealt with in terms of the constant "K". By this means the relation of the "Q-T" interval to the cycle length is concisely given since  $K = "Q-T" - \sqrt{R-R}$ . In the previous study the results were fairly consistent, giving a value of "K" for normal Chinese males in the lying position of  $0.374 \pm 0.012$  and for females  $0.388 \pm 0.0021$ . For the present study a few normal male and female subjects have been added, making a total control series of 116 males and 117 females. The constant "K" remains the same in both but the standard error in females is reduced to 0.0015. Table 1 gives measurements in a normal individual during a 24-hour period, the variations are relatively slight.

For this study 121 male and 100 female patients with congestive heart failure were available. The data are given in Tables 7 and 8. The "Q-T" and "R-R" intervals for these patients and for the 116 normal male and 117 normal female subjects are charted in Figures 1

TABLE 1

*Electrocardiographic measurements of a normal individual (male), during a 24-hour period*

Time	"R-R" interval	"Q-T" interval	"K" *
	seconds	seconds	
9.30 a.m.	0.925	0.350	0.364
10.30 a.m.	0.900	0.358	0.377
11.30 a.m.	0.905	0.355	0.373
12.30 p.m.	1.015	0.360	0.358
2.00 p.m.	0.895	0.360	0.380
3.00 p.m.	0.840	0.335	0.365
4.00 p.m.	0.880	0.340	0.362
5.00 p.m.	0.905	0.360	0.378
9.00 a.m.	0.905	0.355	0.373

$$* K = "Q-T" \text{ interval} \sqrt{R-R \text{ interval}}$$

TABLE 2

*Measurements of examples of records of normal individuals and of patients with heart failure, (all males) (See Fig 3)*

Record	Diagnosis	Degree of heart failure *	R-R Interval	"Q-T" Interval	K †
A	Normal	0	seconds 0.585	seconds 0.284	0.371
B	Mitral disease	III	0.582	0.355	0.465
C	Normal	0	0.660	0.309	0.381
D	Arteriosclerosis Hypertension	I	0.680	0.355	0.431
E	Normal	0	0.579	0.283	0.372
F	Mitral disease	IIa	0.570	0.350	0.464
G	Normal	0	0.620	0.292	0.371
H	Syphilitic	IIb	0.640	0.415	0.514
I	Normal	0	0.856	0.346	0.374
J	Hypertension Arteriosclerosis	III	0.850	0.450	0.489

\* Degree of heart failure is given according to the "Criteria for the Classification and Diagnosis of Heart Disease, 2nd ed., of the New York Tuberculosis and Health Association

$$† K = "Q-T" \text{ interval} \sqrt{R-R \text{ interval}}$$

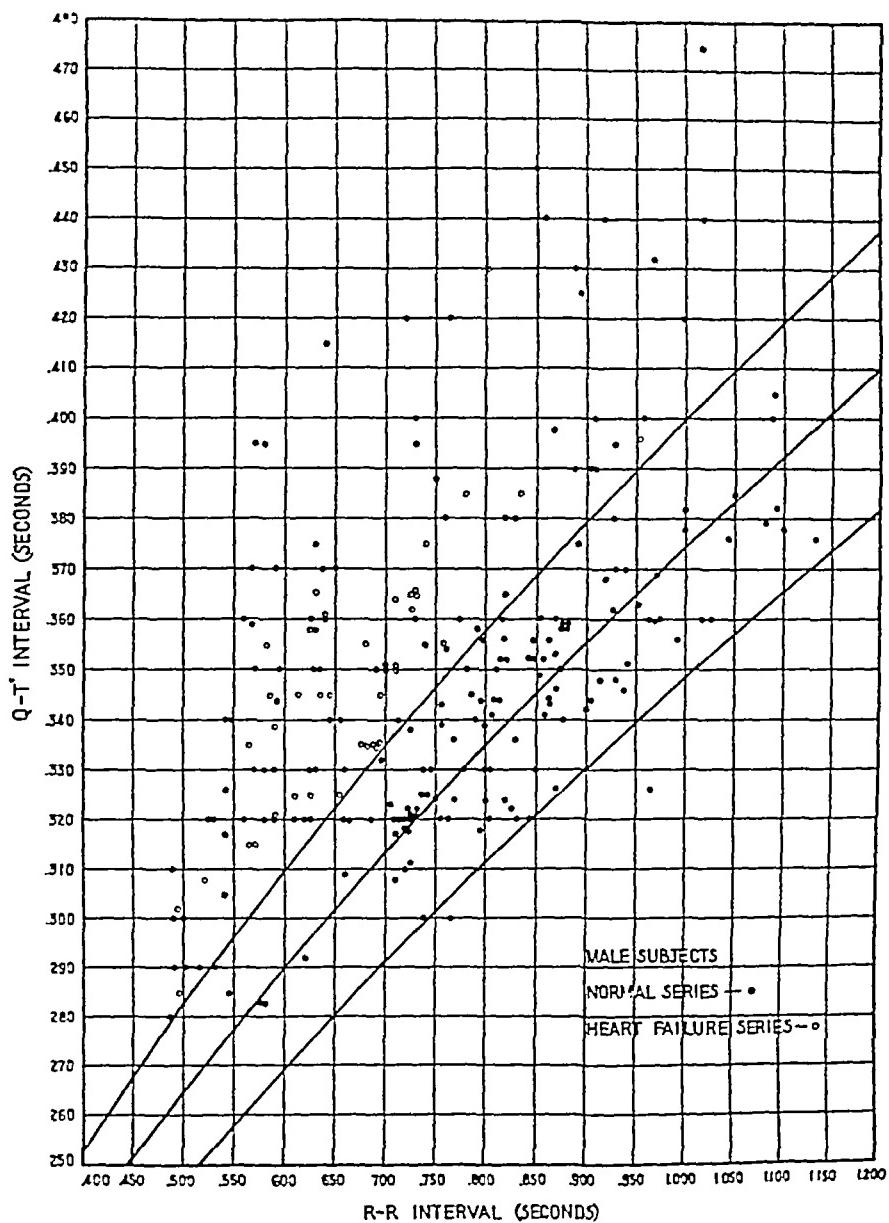


FIG 1 DISTRIBUTION OF "Q-T" AND "R-R" INTERVALS IN THE ELECTROCARDIOGRAMS OF 116 NORMAL MALES AND 121 MALES WITH HEART FAILURE

Central curve is average value (0.374) of "K" ("Q-T" interval / "R-R" interval) for normal males, upper and lower curves are this value plus and minus two standard deviations See Tables 6 and 7

and 2 With few exceptions the value of "K" is large in the patients<sup>1</sup> that is to say, the "Q-T" interval is prolonged out of proportion to the change in the "R-R" interval or cycle length In Figures 1 and 2 the two groups of subjects are fairly well separated (especially in the case of males) Figure 3 and Table 2 illustrate the difference between normal individuals and patients with heart failure in striking instances, in which the cardiac rates are approximately the same Table 3 gives the measurements over several months in two untreated patients, showing the persistence of the relative prolongation of the "Q-T" interval

The results of the statistical study of the measurements made are given in Tables 4 and 5 The correlation between "Q-T" interval and

"R-R" interval of patients with heart failure is high, though not so marked as in normal individuals The difference between the mean values of "K" for normal individuals and for patients with heart failure may be evaluated as follows

	Mean "K" ± standard error
Male patients with failure	$0.432 \pm 0.0023$
Normal male individuals	$0.374 \pm 0.0012$
Difference	$0.058 \pm 0.0026$
Female patients with failure	$0.433 \pm 0.0027$
Normal female individuals	$0.388 \pm 0.0015$
Difference	$0.045 \pm 0.0031$

The differences are clearly statistically significant. There are a number of high values for "K" obtained from the records of women without heart failure. The significance of these data cannot be stated at present

The difference which was found between the values for "K" from records of normal men and women is not found between the values for male and female patients with heart failure (see Table 5)

With regard to the effect of the degree of heart failure,<sup>2</sup> the data as

<sup>1</sup> It has been pointed out that this use of such a constant is not, strictly speaking proper since in patients the original equation can no longer be applied "K" is, however retained for purposes of evaluation and comparison The use of an index of deviation from the normal would needlessly complicate the computations

<sup>2</sup> Heart failure is classified according to the criteria of the Heart Committee of the New York Tuberculosis and Health Association (2)

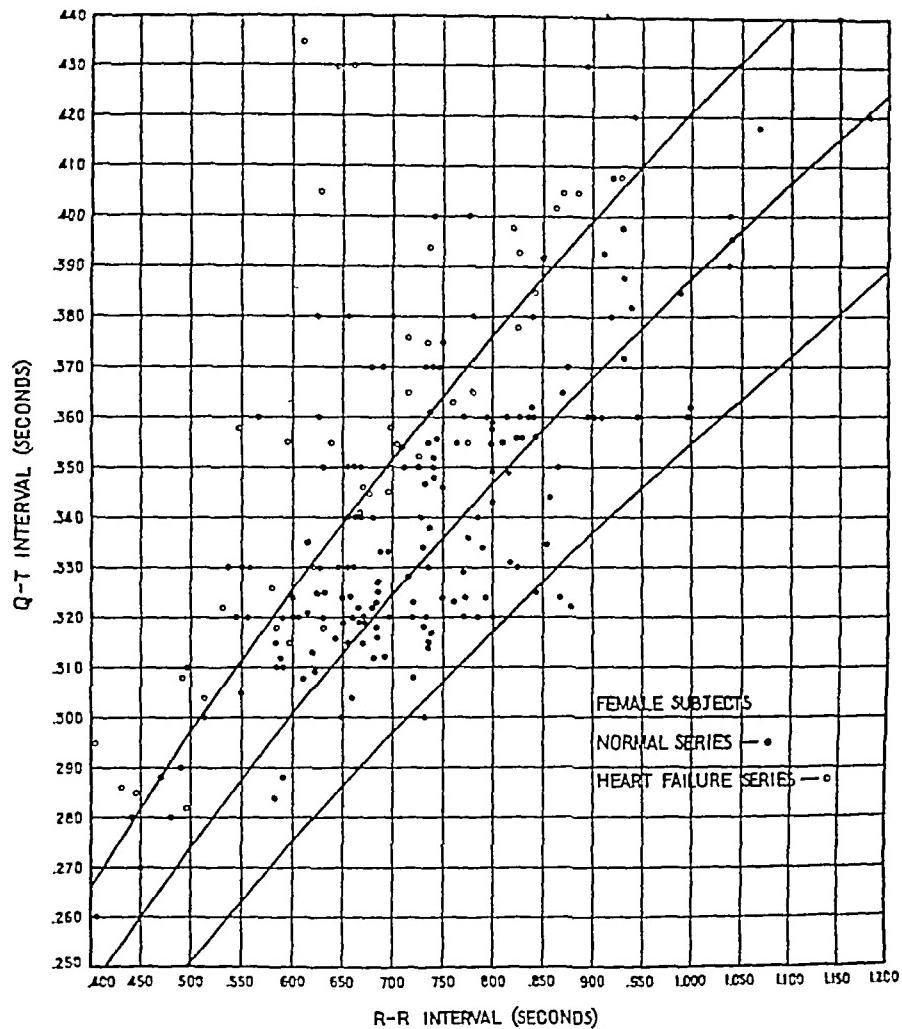


FIG 2 DISTRIBUTION OF "Q-T" AND "R-R" INTERVALS IN THE ELECTROCARDIOGRAMS OF 117 NORMAL FEMALES AND 100 FEMALES WITH HEART FAILURE

Central curve is average value (0.388) of " $K$ " ("Q-T" interval / "R-R" interval) for normal females, upper and lower curves are this value plus and minus two standard deviations See Tables 5 and 8

TABLE 3

*Persistence of relatively long 'Q-T' interval for several months*

Record number, age, and sex	Date	"R-R" interval	"Q-T" interval	K**	Remarks
2566 36 years Male	Nov 25 1929	seconds 0.614	seconds 0.345	0.440	Hypertension Failure IIa
	Nov 29 1929	0.600	0.340	0.439	
	Dec. 2 1929	0.680	0.346	0.420	1.3 gram digitalis in 5 days
	Dec. 30 1929	0.575	0.346	0.456	No treatment
2662 11 years Female	Apr 22 1930	0.575	0.365	0.481	No treatment
	Feb 17 1930	0.445	0.285	0.427	Mitral disease Failure IIb
	July 4 1930	0.505	0.330	0.465	No treatment Failure III
	July 5 1930	0.510	0.350	0.490	No treatment

\* K' = "Q-T" interval  $\sqrt{"R-R" \text{ interval}}$ 

TABLE 4

*Statistical summary of data obtained showing correlation of 'Q-T' and "R-R" intervals in normal subjects and in patients with heart failure*

Group	Number of cases	R-R Interval		Q-T Interval		Correlation coefficient $\pm P.E.$
		Arith. aver $\pm S.E.$	S.D.	Arith. aver $\pm S.E.$	S.D.	
Males with normal hearts	116	seconds 0.836 $\pm$ 0.020	0.1298	seconds 0.342 $\pm$ 0.023	0.0251	0.882 $\pm$ 0.039
Males with heart failure	121	0.678 $\pm$ 0.024	0.1368	0.355 $\pm$ 0.035	0.0394	0.815 $\pm$ 0.026
Females with normal hearts	117	0.772 $\pm$ 0.019	0.1184	0.339 $\pm$ 0.024	0.0262	0.849 $\pm$ 0.0174
Females with heart failure	100	0.670 $\pm$ 0.0132	0.1322	0.352 $\pm$ 0.039	0.0398	0.814 $\pm$ 0.027

Arith aver = arithmetical average S D = standard deviation S E = stand ard error P E = probable error

summarized in Table 6 show only very slight differences, but further work in this direction is necessary. It is however important to note that the relative increase in the 'Q-T' interval is well shown in early heart failure.

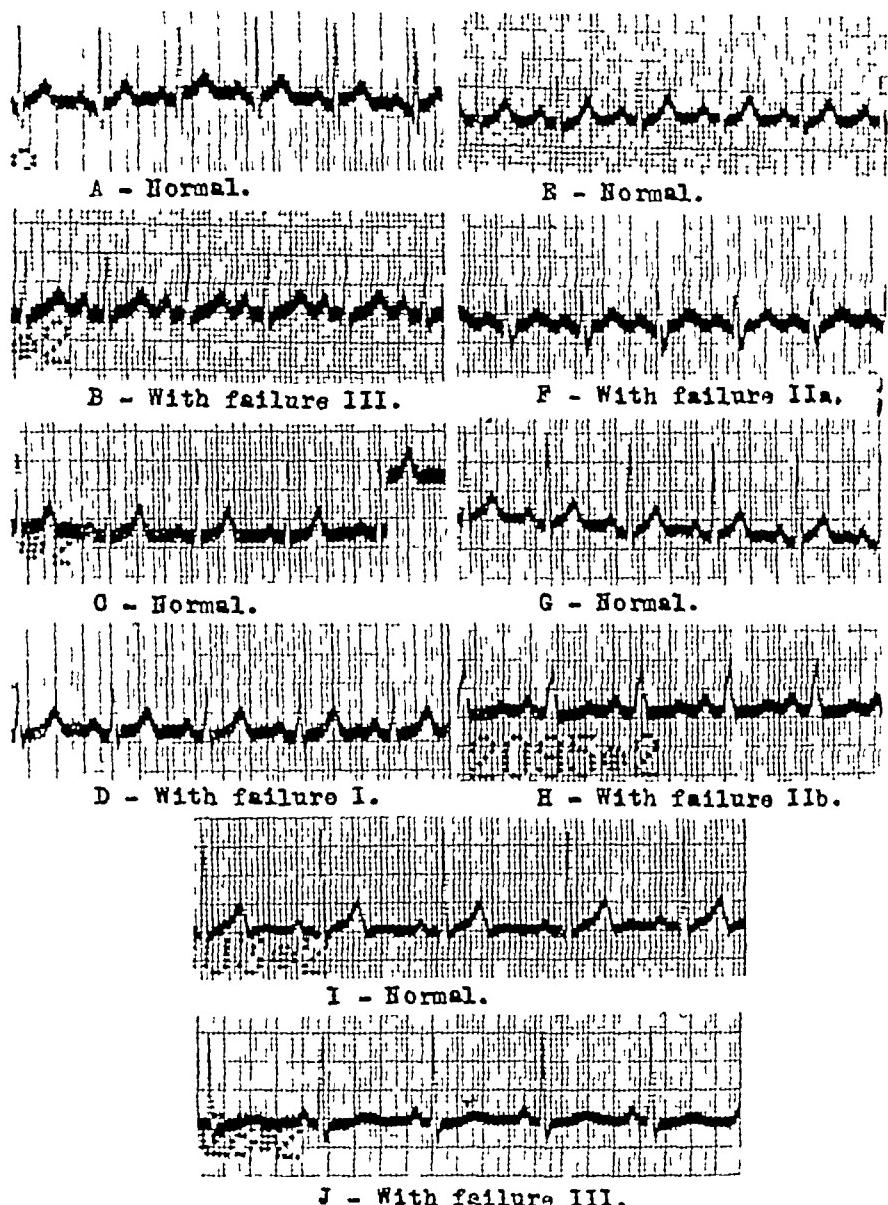


FIG. 3 ELECTROCARDIOGRAMS (LEAD II) OF NORMAL INDIVIDUALS AND INDIVIDUALS WITH HEART FAILURE (SEE TABLE 2)

TABLE 5

The average values of ' $K$ ' for individuals with normal hearts and for individuals with heart failure

Group	Number of cases	$K$ Arith. aver $\pm$ S.E.	S.D.
Normal males	116	0.374 $\pm$ 0.012	0.0129
Males with failure	121	0.432 $\pm$ 0.023	0.0254
Normal females	117	0.388 $\pm$ 0.015	0.0166
Females with failure	100	0.433 $\pm$ 0.027	0.0273
Rheumatic with failure			
Males	43	0.424	
Females	46	0.424	
Males and females	89	0.424 $\pm$ 0.018	0.0174
Syphilitic with failure			
Males	33	0.436	
Females	4	0.435	
Males and females	37	0.436 $\pm$ 0.033	0.0204
Hypertensive with failure			
Males	32	0.441	
Females	37	0.444	
Males and females	69	0.443 $\pm$ 0.076	0.0314
Miscellaneous with failure			
Males	13	0.429	
Females	13	0.431	
Males and females	26	0.430 $\pm$ 0.049	0.0252

Arith aver = arithmetical average    ' $K$ ' = ' $Q-T$ ' interval  $\sqrt{R-R}$  interval  
 S D = standard deviation    S E = standard error

TABLE 6

Summary of average values of ' $K$ ' in patients grouped by degree of heart failure

Degree of heart failure *	Number of cases		$K$ Arith. aver $\pm$ S.E.	$K$ S.D.
	$\sigma$	$\varphi$		
I	19	23	0.423 $\pm$ 0.0031	0.0200
IIa	37	40	0.430 $\pm$ 0.0030	0.0265
IIb	42	18	0.436 $\pm$ 0.0031	0.0243
III	23	19	0.440 $\pm$ 0.0047	0.0307

Arith aver = arithmetical average    S D = standard deviation    S E = stand ard error    ' $K$ ' = ' $Q-T$ ' interval  $\sqrt{R-R}$  interval

\* Degree of heart failure is given according to the "Criteria for the Classification and Diagnosis of Heart Disease" 2nd ed of the New York Tuberculosis and Health Association

## DURATION OF ELECTRICAL SYSTOLE

TABLE 7  
*Clinical and electrocardiographic data for 121 males with heart failure*

ABBREVIATIONS			
AID	= aortic disease (stenosis and regurgitation)	G A	= general arteriosclerosis
Af	= atrioventricular fibrillation	H	= hypertension
AR	= aortic regurgitation	L V P	= left ventricular preponderance
Ac Neph	= acute nephritis	M D	= mitral disease (stenosis and regurgitation)
B B B	= bundle branch block	P T b	= pulmonary tuberculosis
Bact Endo	= subacute bacterial endocarditis	R V P	= right ventricular preponderance
Chr Neph	= chronic nephritis	Ret Art	= retinal arteriosclerosis
Cong Ht Dis	= congenital heart disease	S	= syphilis
Cor Ob	= coronary obstruction	S C N S	= syphilis of central nervous system

I h G number	Age	Clinical diagnosis*	Heart over size square cm	Degree of heart failure†	Blood pressure mm Hg	P-R interval seconds	R-R interval seconds	Q-T interval seconds	A ‡	EKG remarks
A RHEUMATIC HEART DISEASE										
2097	27	M D, A D, A F	77	III	120/77	?	580	395	430	R V P, V T
1039	36	M D, A D	74	I	160/68	17	910	400	419	L V P
855	19	? M D, A D	38	II <sup>a</sup>	116/38	20	630	375	472	L V P
1105	18	M D, A D	77	IIb	114/20	20	580	330	433	L V P
1626	35	M D	41	I	100/80	15	930	395	415	R V P
1620	10	M D, Lymphema	42	IIb	108/76	16	645	340	423	R V P
1270	35	M D	*	IIb	134/68	19	590	330	429	Normal
1218	26	M D	56	IIb	92/64	16	570	350	464	R V P
586	29	? M D	*	IIb	120/85	16	690	335	401	Normal
1147	27	M D, A D	38	II <sup>a</sup>	138/58	27	620	320	406	Normal
1897	16	M D, A D, P r b	30	II <sup>a</sup>	128/0	20	530	290	400	Normal
1903	38	M D	16	I	120/61	16	655	396	405	I V P

TABLE 7 (continued)

E.K.G. number	Age years	Clinical diagnosis	Heart over size square cm	Degree of heart failure †	Blood pressure mm Hg	P-R" interval seconds	R-R" interval seconds	Q-T interval seconds	K"‡	E.K.G. remarks
1921	34	M.D., S	Normal	IIa	100/56	14	692	.350	423	R.V.P
1750	27	M.D.	17	I	105/50	17	893	.375	399	Normal
1686	16	M.D.	4	IIa	112/78	16	730	.365	427	Normal
1628	15	M.D., A.D	60	IIb	92/28	17	.570	.315	417	L.V.P
1907	56	?M.D.	21	IIb	90/68	16	.595	.350	454	L.V.P
1580	36	M.D.	40	IIb	115/75	18	655	.340	420	Normal
1886	19	M.D.	6	IIa	80/40	20	680	.335	406	L.V.P
1899	35	Acute carditis, ?M.D	26	IIa	102/24	17	630	.330	416	Normal
1369	26	M.D., A.F	55	IIa	90/7	?	.592	.344	447	R.V.P A.F
1764	31	M.D., A.F	*	IIb	100/?	?	.545	.340	460	R.V.P A.F
2207	33	M.D., S	79	III	94/64	18	625	.330	419	B.B.B
1725c	21	M.D., A.D., S	21	III	98/48	20	.582	.355	465	Normal
2717	28	M.D., P.T.b	42	IIa	116/70	13	490	.290	414	R.V.P
346	24	M.D., ?A.D	104	IIa	110/88	16	695	.335	402	Normal
1917	40	M.D.	*	I	100/70	17	780	.350	.398	R.V.P
2681a	29	M.D.	*	IIa	100/60	16	688	.335	404	Normal
2390	13	M.D.	*	IIb	?	16	490	.310	443	L.V.P
1390	16	M.D.	*	IIa	90/75	18	625	.320	405	R.V.P
2245	22	M.D.	*	IIa	95/80	17	655	.325	402	R.V.P
2340	22	A.D.	42	I	110/40	20	792	.358	402	L.V.P
2344	30	M.D. Emphysema	*	IIb	100/60	16	750	.388	447	R.V.P
2659	19	M.D., A.D.	86	I	130/30	20	758	.355	408	Normal
1714	15	M.D.	19	I	94/54	.21	710	.350	415	Normal
2865	41	A.D.	46	IIa	95/50	.28	640	.360	450	L.V.P
2903	21	A.D.	73	III	120/60	16	.525	.320	441	R.V.P

TABLE 7 (continued)

I. h. G. number	Age (years)	Clinical diagnosis		Heart over size square cm	Degree of heart failure †	'P-R' interval seconds	'R-R' interval seconds	Q-T interval seconds	A ‡	EKG remarks
		M.D.	S							
2909	36	M.D., S		75	III	114/76	18	560	128	R V P
3053	21	M.D., A.D		*	III	100/20	16	625	153	Normal
2201q	34	M.D.		67	IIb	90/70	20	970	132	Normal
2780	23	M.D., A.D		62	I	120/15	20	960	108	Normal
2781	11	M.D.		*	IIa	78/50	16	590	320	Normal
2876	28	M.D.		4	III	106/80	16	496	285	R V P
<b>B SYPHILITIC HEART DISEASE</b>										
1570	16	S, G.A., II		*	IIb	186/98	16	566	370	V P
1510	58	S, A.R., Pneumonia		*	IIb	145/35	16	635	315	V P
1362b	57	S, Myocarditis		*	IIb	118/70	17	820	380	L V P
1130	38	S, A.R		45	IIb	128/28	17	640	415	Normal
782	19	S, G.A., II		88	IIb	165/90	16	645	345	L V P
1153	58	S, A.R		*	IIb	128/40	16	630	350	L V P
2051	38	S, A.R		41	IIb	116/40	19	626	325	III Normal
1861	61	S, G.A.		*	IIa	172/42	16	585	315	R V P
1911	41	S, Aortitis, S C N S		5	IIa	124/50	16	780	385	L V P
2431	45	S, A.R		*	IIa	149/40	11	520	308	127 L V P
2084	59	S, Aortitis, Angina		4	IIa	120/78	16	495	302	429 L V P
2055e	60	S, A.R		*	IIa	145/55	16	765	420	434 L V P
2011	46	S, A.R		*	IIb	116/46	17	560	360	481 Normal
2709	10	S, A.R., P T b		32	IIa	90/50	16	565	315	I V P
2583	38	S, Gummari of heart		*	III	68/56	16	512	326	143 I V P
2831	56	S, A.R., Aortitis		66	III	146/68	16	530	320	410 I V P
3033	11	S, S C N S, II		1	IIa	152/90	16	685	335	105 Normal

TABLE 7 (continued)

E.K.G. number	Age	Clinical diagnosis*	Heart over size	Degree of heart failure†	Blood pressure	"P-R interval, seconds"	R-R interval, seconds	Q-T interval, seconds	K ‡	E.K.G. remarks
2782	51	S Cirrhosis of liver	*	I	90/45	20	820	365	403	L V P
2819	50	S A.R.	*	IIb	120/30	16	730	360	421	Normal
2677	44	S A.R.	*	IIa	192/30	16	610	325	416	Normal
1672	48	S A.R.	118	IIb	156/80	16	869	398	427	L V P
2563	54	S, Aortitis, Cor Ob	*	III	96/68	17	548	340	460	L V P
2618	54	S, A.R.	63	IIa	140/50	20	730	365	427	B B B
2776	41	S, A.R.	*	III	135/30	15	630	358	451	L V P
2655	60	S, A.R.	54	IIb	180/78	16	740	355	413	L V P
2778	43	S, A.R.	16	I	100/45	16	638	370	463	Normal
2855	44	S, A.R.	24	IIb	152/46	16	700	350	418	L V P
2879	45	S, A.R.	47	IIb	120/40	13	590	320	416	Normal
2965	41	S, A.R.	30	I	110/58	16	760	380	436	L V P
3056	61	S, Aortic aneurism	17	IIa	134/60	16	890	420	445	L V P
3012	45	S, A.R.	*	III	128/44	15	541	317	431	L V P
2501	27	S, A.R.	15	III	102/38	17	710	364	432	L V P
2539	46	S, A.R.	60	III	190/20	16	726	362	425	B B B
C HYPERTENSIVE AND ARTERIOSCLEROTIC HEART DISEASE										
1649	28	II Chr Neph	73	IIb	200/130	14	490	300	428	L V P
1670	49	H G A Chr Neph	16	IIa	200/100	16	910	390	409	R. V P
1441d	58	H G.A. S	38	IIb	150/100	17	730	400	468	L V P
1406	23	H, Ac. Neph	26	IIb	180/135	14	395	260	414	L V P
1815	53	H Chr Neph Bronchopneumonia	*	III	196/120	16	540	305	415	L V P
1779	55	II, S	*	III	184/100	28	920	440	459	B B B

TABLE 7 (continued)

E.K.G. number	Age years	Clinical diagnosis	Heart over size square cm	Degree of heart failure †	Blood pressure mm Hg	"P-R" interval seconds	"R-R" interval seconds	"Q-T" interval seconds	K.‡	Γ K.G. remarks
1893	52	H, GA, Chr Neph	III	III	214/136	1.4	725	365	429	Normal
1350	47	H, GA, Chr Neph	IIb	IIb	175/140	1.6	650	370	459	L V P
2221	60	H, Chr Neph	*	IIb	145/105	1.6	570	330	437	L V P
2545	36	H, Ac Neph	35	IIb	174/110	1.5	860	410	474	Normal
2648	53	H, GA, Chr Neph	2	IIa	210/120	1.6	630	365	460	L V P
2122	59	H, GA	29	I	183/110	1.6	680	355	431	R V P
2712	27	Chr Neph	43	I	128/84	1.6	910	390	409	L V P
1241	17	H, Chr Neph	4	I	164/92	1.7	1020	440	436	Normal
1919	55	H, GA	*	IIb	140/80	1.6	580	320	421	I V P
1692	26	Chr Neph, Uremia	19	III	128/80	1.5	850	450	489	I V P
3886	65	H, GA, A.R.	*	III	150/60	1.8	835	385	421	L V P
191a	63	H, GA, A.R.	*	IIa	164/66	1.6	1020	475	470	L V P
2110	51	H, GA, Chr Neph	*	IIb	144/68	1.3	590	370	482	L V P
2695	52	H, GA, Hemiplegia	*	IIa	150/90	1.6	997	420	421	B B B
2638	49	H	23	IIa	154/90	1.9	720	420	495	B B B
2694	70	G A, Emphysema, Bronchietasis	35	IIb	108/70	1.6	565	335	446	R V P
2836	62	H, GA, A.R.	54	IIa	160/20	2.8	930	380	394	I V P
2186	46	H	*	I	150/98	2.0	890	390	415	R V P
2566	36	H, GA, Ret Art	28	IIa	230/160	1.6	614	345	440	I V P
2916	45	H, Angina pectoris	*	IIa	150/100	1.6	610	320	410	I V P
2528	18	H	*	I	170/90	2.0	730	395	463	L V P
2888f	62	H, GA	80	III	155/110	1.6	700	350	418	L V P
2817	41	H, Chr Neph, Bronchopneumonia	*	IIb	150/120	1.6	515	290	404	I V P

TABLE 7 (concluded)

E.K.G. number	Age	Clinical diagnosis	Heart over size spare cm.	Degree of heart failure †	"P-R" interval msec. Hertz	"R-R" interval seconds	"Q-T" interval seconds	K ‡	E.K.G. remarks
2921	52	H G.A. Chr Neph. Bronchopneumonia	*	III IIb IIa	200/110 195/130 180/108	.16 .16 .15	.570 .710 .895	.395 .350 .425	Normal L.V.P. Normal
2934y	42	H Chr Neph	51						
2961	30	H, Chr Neph	24						
<b>D MISCELLANEOUS CASES</b>									
2399	26	Septic pericarditis	84	IIb	84/40	.15	.566	.359	477 Normal
2572	23	M D A.D., Bact. Endo Cerebral embolism	23	IIa	126/28	.18	.695	.345	414 L V P
684	19	M D, A.D. Bact Endo	24	IIa	110/40	.16	.500	.300	425 R.V.P.
1100	61	Emphysema	60	III	130/70	.17	.626	.360	455 Normal
2010	39	Emphysema H	55	IIb	122/98	.16	.660	.330	406 Normal
2557	17	Cong. Ht. Dis.	10	IIa	104/80	.15	.590	.339	442 R.V.P.
1123	20	Beriberi	22	IIb	112/50	.16	.775	.360	409 Normal
1072	23	Beriberi	112	IIb	98/50	.16	.830	.380	417 Normal
2280	18	Beriberi	50	IIb	120/50	.16	.485	.280	402 R.V.P.
1125	13	Ac. failure cause?	*	III	120/80	.13	.502	.290	409 Normal
2621	61	Asthma Emphysema	*	IIa	108/60	.23	.740	.375	436 L.V.P.
2939	23	Cong. Ht. Dis.	Normal	I	90/70	.16	.635	.350	440 Normal
2950	38	Cong. Ht. Dis.	43	I	90/60	.16	.640	.360	451 L V P

\* Heart size is given from teleoentgenograms. In cases marked thus (\*), measurement could not be made satisfactorily but the heart was enlarged, judged by the general appearance of the teleoroentgenogram and by physical examination.

† Degree of heart failure is given according to the "Criteria for the Classification and Diagnosis of Heart Disease," 2nd ed. of the New York Tuberculosis and Health Association

‡ "K" = "Q-T" interval  $\sqrt{"R-R"}$  interval

## DURATION OF ELECTRICAL Systole

TABLE 8  
*Clinical and electrocardiographic data for 100 females with heart failure  
 (Abbreviations as in Table 7)*

I K G number	Age years	Clinical diagnosis	Heart size square cm	Degree of heart failure †	Blood pressure mm Hg	P-R interval seconds	R-R interval seconds	'Q-T' interval seconds	K ‡	I K G remARKS
171	37	M D , S	*	IIa	96/64	18	780	380	131	R V P
180f	38	M D , A D	.47	IIa	95/50	20	850	392	125	R V P
2110	39	M D , A D	5	I	82/40	16	940	420	133	I V P
1786	15	M D	107	III	102/78	16	490	290	414	Normal
2310	27	M D , S	*	IIb	96/60	16	620	330	119	R V P
2078j	47	M D	*	IIa	110/70	15	735	394	160	R V P
1932g	12	M D , Pericarditis, II	*	IIa	140/100	20	699	358	429	R V P
1809	36	M D , S , II	66	IIa	140/120	20	655	340	414	R V P
1715	37	M D , Bronchopneumonia	*	IIb	90/70	14	470	288	420	R V P
1963	52	M D , Secondary anemia	75	III	102/48	16	842	385	420	Normal
1903	12	M D , Bronchopneumonia	81	IIb	112/86	19	530	322	111	R V P
609k	21	M D , S	*	III	90/60	18	590	310	404	R V P
1171	47	M D , A Γ , Pericarditis	*	IIb	70?	?	512	304	130	R V P , I
870	25	M D , Pericarditis	117	III	88/68	16	497	310	439	R V P
1126	20	M D , Pregnancy	111	III	90/60	16	598	315	110	R V P
1427i	19	M D	60	III	100/60	13	560	320	428	Normal
2785	20	M D , A Γ	*	I	108?	?	410	260	106	R V P , A I
1587a	26	M D	108	I	110/68	21	1150	110	110	R V P
66f	25	M D	*	IIa	100/60	14	725	350	111	Normal
331b	13	M D	8	IIa	100/75	16	820	398	439	R V P
1859	25	M D	*	IIa	100/60	16	710	350	115	R V P

TABLE 8 (continued)

R.E.G. number	Age	Clinical diagnosis	Heart over size sq m.	Degree of heart failure †	Blood pressure mm. Hg	P-R interval seconds	R-R interval seconds	Q-T' interval seconds	X ‡	E.K.G. remarks
2662	10	M.D	Ia	126/76	18	445	285	427	R.V.P	
2095	35	M.D	IIb	120/80	17	662	350	430	R.V.P	
2312a	36	M.D, A.D	IIb	110/60	16	861	402	433	Normal	
2663	42	M.D, A.D H	IIa	120/100	16	810	355	394	Normal	
2307	28	M.D A.D H	IIa	135/95	16	732	370	433	Normal	
2734	26	M.D, A.D	III	110/40	17	625	325	411	Normal	
2813	18	M.D	IIa	80/58	18	840	380	415	R.V.P	
2799	22	M.D	IIa	96/60	15	605	320	411	R.V.P	
2873a	25	M.D	IIa	110/70	16	700	380	454	R.V.P	
2874	46	M.D, A.F, S. Cerebral embo	25	I	100/R	?	745	370	429	R.V.P A.F
2918	24	M.D, Pericarditis	82	III	124/70	28	630	350	441	L.B.B.B
2931	37	M.D	IIa	110/70	16	670	346	423	Normal	
2853	35	M.D	II	100/80	16	440	280	422	Normal	
3015	25	M.D, Pregnancy	IIa	82/60	18	590	320	416	R.V.P	
2744b	31	M.D	IIb	100/70	16	638	355	444	R.V.P	
2679	56	M.D A.F	89	IIb	150/R	?	760	363	416	A.F
2549	22	A.D, ? M.D	43	I	130/60	15	770	360	411	L.V.P
2427	26	M.D	14	IIa	100/80	20	710	354	420	Normal
2550a	33	N.D	*	IIa	90/60	13	670	350	428	L.V.P
2551	31	M.D Bronchopneumonia	92	III	110/80	20	580	326	428	R.V.P
2315	47	M.D A.D, G.A	26	I	170/80	20	775	355	405	L.V.P
2856	23	A.R. Bact Endo	*	III	124/14	08-48	690	370	445	H.B
2298	39	M.D, A.D	*	I	100/70	16	870	405	434	R.V.P
2615	16	M.D	*	IIa	95/40	20	675	345	420	Normal
2656	39	M.D	18	IIa	106/66	14	630	318	401	Normal

TABLE 8 (continued)

Gamma Kappa G number	Age	Clinical diagnosis	Heart over size square cm.	Degree of heart failure †	Blood pressure mm Hg	P-R' interval seconds	R-R' interval seconds	Q-T interval seconds	K ‡	Gamma Kappa G remarks
<b>B SYPHILITIC HEART DISEASE</b>										
2975	16	S, A.R., II	*	III	210/100 135/50	16	750	400	465	L V P
1520	63	S, A.R.	18	I	125/30	16	825	393	433	Normal
1916	17	S, A.R.	*	I	130/80	18	920	408	426	L V P
2671	15	S, Aortitis	15	IIa	130/80	17	735	355	114	Normal
<b>C HYPERTENSIVE AND ARTERIOSCLEROTIC HEART DISEASE</b>										
1983	23	H, Ac Neph	*	III	240/170	12	495	282	401	Normal
1035	37	H, Chr Neph, Neuroretinitis	23	IIa	210/125	15	655	380	469	Normal
1191	48	H, Chr Neph, Obesity	27	IIb	168/100	17	617	335	127	R V P
1559	51	H, Chr Neph, S	*	IIa	230/102	16	928	408	124	Normal
2385	14	H, Ac Neph, Tonsillitis	17	IIa	170/100	16	698	350	432	R V P
2012	63	H, G \, Diabetes	*	III	168/94	16	625	380	181	Normal
2343	43	H	*	IIa	170/90	16	730	376	110	L V P
1777	51	H, G A	*	IIa	250/130	16	738	361	421	Normal
2369	42	H	*	IIa	168/110	16	735	375	438	B B B
1785	39	H, S	*	IIb	145/98	16	738	370	431	Normal
2325	37	H, Ac Neph, Tonsillitis	*	IIa	160/110	16	655	324	400	Normal
2698	13	H, Chr Neph	*	IIa	260/140	15	665	310	417	Normal
2327a	42	H	*	IIa	144/90	16	645	345	429	L V P
2666	37	H, Chr Neph	20	IIa	200/140	16	548	358	484	Normal
2643	43	H, G A, Chr Neph, S	42	IIb	200/120	16	625	360	455	Normal
1068	66	H, G A, A.F, Pyonephrosis	*	IIb	160/72	2	430	286	428	L V P, A F
1250	66	G A	*	IIa	130/60	16	885	405	132	Normal
1909a	57	G A, A F	*	III	132/72	?	600	325	420	A I

TABLE 8 (continued)

E.K.G. number	Age	Clinical diagnosis	Height over size square cm	Degree of heart failure †	Blood pressure mm. Hg	"P-R interval seconds"	"R-R interval seconds"	"Q-T interval seconds"	K ‡	E.K.G. remarks
2690 37	H	Chr Neph	*	IIb	160/100	.16	.615	.330	.420	L V P
1890 48	H, G.A.	G.A.	*	I	150/80	.16	.795	.360	.404	L V P
2083 49	H	G.A.	*	I	170/75	.20	.930	.398	.413	Normal
2118 42	G.A.	Chr Neph	*	IIa	120/70	.16	.660	.350	.431	Normal
2839 43	H	G.A. S Ret. Art	*	IIa	180/110	.16	.660	.430	.530	Normal
2866 47	G.A.		26	III	130/70	.04	.405	.295	.464	Auricular tachycardia
2893 52	H	G.A.	*	III	280/160	.16	.645	.430	.533	L V P
2894 45	H		*	I	140/110	.18	.680	.370	.449	Normal
2914 66	G.A. A.R.	Coronary sclerosis	23	IIb	130/80	.20	.775	.400	.454	Normal
2945 60	H	G.A.	*	IIa	215/120	.14	.480	.280	.436	L V P
2686 33	H	G.A. H Ret Art	*	I	140/110	.16	.715	.365	.432	L V P
2290 40	H	Hemiplegia, Ret Art	*	I	160/120	.13	.728	.352	.413	L V P
2562 47	H	Angina pectoris	*	III	165/105	.18	.705	.355	.423	L V P
2552 48	H		*	I	150/90	.13	.825	.378	.416	Normal
2722 45	H	Obesity	Normal	I	150/90	.16	.565	.360	.479	L V P
2165 60	H		*	IIa	240/100	.16	.895	.430	.454	B B B
2705 43	H		*	I	220/120	.16	.750	.375	.433	L V P
2371 47	H	? M D	*	IIa	220/120	.15	.610	.435	.553	Normal
2733 17	H	Chr Neph	Normal	I	218/150	.15	.595	.355	.460	L V P
<b>D MISCELLANEOUS CASES</b>										
1185 23	P T b	T b of pericardium	198	IIb	94/65	.16	.510	.300	.420	R. V P
1089 24	"	"	79	I	99/50	.16	.450	.270	.403	Normal
1030 24			*	IIb	98/70	.14	.647	.330	.410	Normal

## DURATION OF ELECTRICAL SYSTOLE

TABLE 8 (concluded)

R. K. G. number	Age	Clinical diagnosis	Heart over size square cm	Degree of heart failure †	Blood pressure mm Hg	P-R interval seconds	"R-T" interval seconds	Q-T interval seconds	K ‡	R-K G remark*
1398	25	M D , Bact Endo , Cerebral embolism	*	I	110/70	20	58.5	310	405	Normal
2016	55	Cholecystitis, Bronchopneumonia	*	IIIb	95/35	18	54.0	320	135	I V P
2279	10	II , Myocarditis	7	III	132/92	12	62.8	105	512	B B B
496	11	Myocarditis	*	IIa	115/80	16	78.0	365	111	R V P
2694	25	Hyperthyroidism	1.4	IIa	118/55	20	55.5	330	443	I V P
2792	33	Hyperthyroidism	21	IIa	130/60	18	55.0	305	111	Normal
2811	39	Hyperthyroidism	4	IIa	125/65	20	55.0	330	145	Normal
2846	30	M D , Bact Endo , Hemiplegia	49	IIa	90/60	20	53.5	330	451	R V P
2428	46	Hyperthyroidism	*	I	130/65	16	49.0	308	440	Normal
2326	19	Cong II Dis	*	I	100/40	13	58.4	318	116	Normal

\* Heart size is given from teleroentgenograms. In cases marked thus, (\*), measurement could not be made satisfactorily but the heart was enlarged, judged by the general appearance of the teleroentgenogram and by physical examination.

† Degree of heart failure is given according to the "Criteria for the Classification and Diagnosis of Heart Disease," 2nd ed of the New York Tuberculosis and Health Association.

$$\sqrt{\text{"R-R" interval}}$$

‡ "K" = "Q-T" interval

The nature of the underlying heart disease might be thought to play an important rôle in this connection, though this is not necessarily true. Table 5 shows the average values of "K" obtained from records of individuals with heart failure grouped by etiology. The values may be compared as follows:

	Mean "K" ± standard error
Hypertensive group	0.443 ± 0.076
'Rheumatic' group	0.424 ± 0.018
Difference	0.019 ± 0.078
Syphilitic group	0.436 ± 0.033
'Rheumatic' group	0.424 ± 0.018
Difference	0.012 ± 0.037
Miscellaneous group	0.430 ± 0.049
Rheumatic group	0.424 ± 0.018
Difference	0.006 ± 0.052

The values of "K" for the hypertensive and syphilitic groups are slightly higher than the values for the rheumatic group, but the difference is hardly significant. If the difference should prove significant, it would accord with the clinical impression that rheumatic heart disease is often better borne than manifest hypertensive or syphilitic heart disease. No other significant difference is suggested by these data.

No clear correlation between size of heart as determined by tele-roentgenograms measured by the method of Hodges and Eyster (3) and the value of "K" has been found nor can any statement be made with regard to the effect of acute dilatation because the only cases available have been necessarily complicated by digitalis therapy.

No constant association between changes in the electrocardiogram such as prolonged condition time, bundle branch block, or ventricular preponderance and the size of "K" was discernible.

In a further study soon to be published the effect of digitalis upon the value of "K" has been observed to be that of fairly consistent reduction.

#### DISCUSSION

The data presented seem to warrant the conclusion that in the presence of manifest heart failure the "Q-T" interval of the electro-

cardiogram is prolonged beyond normal limits, taking into consideration the heart rate. It does not follow that this is the only circumstance in which the phenomenon occurs. In fact it has been found in the present study that some individuals with anatomic damage to the cardiovascular system have large values for "K," though no heart failure is discernible. But one must remember that when clinical symptoms and signs of congestive heart failure are present, myocardial exhaustion must be relatively advanced. It is reasonable to suppose that minor changes in the functions of the ventricles must first take place. Indeed it is noteworthy that grave myocardial exhaustion may occur without objective signs of congestive failure such as edema, ascites, or enlarged liver.

The significance of the "Q-T" interval has been a disputed point. Feil and Katz (4) found by measurements of heart sound and central pulse records that of 13 patients with hypertension and heart failure six showed an abnormally short systole and only one an abnormal lengthening. White and Mudd (5) have recently studied the "Q-T" interval in a large number of pathological conditions and concluded that its measurement was apparently of little or no clinical value. In neither of these reports was particular attention paid to various factors that may influence the "Q-T" interval, such as sex, temperature, and digitalis, nor were statistical methods used.

A summary of the situation in regard to normal conditions is given in the paper previously referred to (1). For reasons there presented and because of the high degree of correlation shown in Table 4 and Figures 1 and 2, it seems to the authors justifiable to conclude that the "Q-T" interval has a significant direct relation to cardiac systole. This may be true with regard to pathological conditions as compared with normal conditions, even though there are normally independent variations, provided the normal variations are within a certain limit. In case the "Q-T" interval is in this sense directly related to the length of cardiac systole, the effect of the phenomenon here described upon the output of the heart would be important. No data on this subject are available. From the cardiodynamic point of view, an increased systolic discharge is accomplished in part through an increase in velocity of ejection and in part by a prolongation of systole in the presence of increased venous return (Wiggers (6)).

The prolongation of systole does encroach upon the duration of diastole, and thus diastole becomes shorter in a rapid failing heart. But the systolic discharge depends on the rate as well as on the duration of diastolic filling. In a rapid heart with an increased venous pressure, though the duration of diastole may be much shortened, the systolic discharge may still remain unchanged or may even be increased due to increased rate of diastolic filling which Wiggers (6) found to occur experimentally in dogs. Hence the prolongation of systole at the expense of diastole may not necessarily indicate a decreased output in the presence of increased venous pressure unless the action of the myocardium is seriously interfered with by disease. The current view of heart failure has been, however, that the output of the ventricles is reduced and there is not a little evidence pointing in this direction.

Practically nothing is known about factors that modify the duration of systole in human beings except that it varies with cycle length in normal individuals (see references in (1)). On the basis of experiments in animals it is believed that the conditions which are associated with an increase in systolic length are 1 Increased venous return or diastolic volume, 2 Continued vagus stimulation, and 3 Increased arterial resistance. In regard to the last point opinions vary. According to Patterson, Piper, and Starling (7) prolongation occurs, while Wiggers (8) found it only when the resistance is placed at the aorta. Cardiac dilatation or increase of systolic and diastolic volume and increased venous pressure are present in all cases of congestive failure and undoubtedly exaggerate the relative prolongation of systole. As to the vagus effect, we have practically eliminated it by the atropin test. Atropin, 0.002 gram, was given to a few patients with cardiac failure and prolonged systole. No appreciable reduction of systolic duration occurred.

The relative prolongation of "Q-T" interval in cardiac insufficiency seems to be of myocardial origin. Whether it is a reaction of the myocardium to the factors that brought about its failure, or a manifestation of fatigue of the myocardium is a problem for further study.

The results of Katz (9) with regard to mechanical systole in dogs must be mentioned. Katz found that mechanical systole in dogs was shortened before signs of heart failure appeared. These changes were produced by rapid increase in venous pressure and resulted in acute

dilatation of the heart. Acute cardiac dilatation clinically differs much from slowly progressive failure and the experiments are hardly comparable to the events of human clinical pathology.

On the hypothesis that the "Q-T" interval is directly related to ventricular systole within the limits set forth above, the prolongation here described is an important fact with regard to the dynamics of heart failure in man. In any case, if established, it is a phenomenon of independent interest.

#### SUMMARY

1 In cardiac failure resulting from various causes the electrical systole ("Q-T" interval of the electrocardiogram) has been found prolonged beyond normal limits, taking into consideration the rate of the heart. This is expressed in the value of "K" which is the ratio, systole ("Q-T" interval) square root of cycle length ("R-R" interval).

2 No direct relation between degree of heart failure and the value of "K" was found, but relative prolongation of the "Q-T" interval is present in the earliest recognizable cases of myocardial insufficiency.

3 In rheumatic heart disease the value of "K" is not as high as in hypertensive and syphilitic cardiovascular disease.

4 Electrocardiographic abnormalities such as prolonged conduction time and ventricular preponderance did not obviously effect the value of "K" nor was any correlation between value of "K" and size of heart seen.

5 The factors that may be responsible for the relative prolongation of the "Q-T" interval in heart failure are discussed.

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# THE EFFECTS OF SUSTAINED PITUITARY ANTIDIURESIS AND FORCED WATER DRINKING IN EPILEPTIC CHILDREN A DIAGNOSTIC AND ETOIOLOGIC STUDY<sup>1</sup>

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In the course of work already reported (1) (2) on the relationship of water balance to the occurrence of convulsions in severely epileptic children, it was found that seizures, after having been brought under control by the production of a deficit in the body water, could be made to recur practically at will by the rapid reestablishment of a positive water balance with pituitary antidiuresis. The investigation reported in the present paper was undertaken with the purpose of further elucidating the mechanism of this reaction which appeared to us to open a new avenue of approach to the complex problem of etiology in epilepsy.

In order to avoid misinterpretations based upon the spontaneous occurrence of seizures, patients were selected who were known to have convulsions infrequently but with a fair degree of regularity. It was believed that any fundamental difference that might be found between the response of the normal subject to an artificially induced disturbance in the body fluid relationships and that of the epileptic patient during his usual period of freedom from seizures, might furnish valuable information regarding the underlying physicochemical pathology in epilepsy. The possibility of utilizing the observed reaction of the severe epileptic to an enforced positive water balance as a diagnostic procedure in mild or obscure cases also occurred to us early in the study. Other means by which seizures can often be provoked in severe epi-

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lepsy, such as hyperventillation of the lungs (3) and the production of marked alkalosis from ingestion of sodium bicarbonate (4), are rarely effective in the mild cases during the intervals between their infrequent seizures. This observation led McQuarrie and Keith (5) to conclude that the mildly epileptic patient is indistinguishable from the normal child during his free periods. It will be seen, however, that the results presented here necessitate a reversal of that opinion.

#### PLAN OF INVESTIGATION

The experimental subjects consisted of three groups of children. The first was made up of patients who gave personal histories more or less typical of "idiopathic" epilepsy, but had seizures at infrequent intervals. The second included cases with very indefinite histories of "fainting spells" or "sleepy spells" but no history of generalized convulsions or true *petit mal*. The third group consisted of essentially normal children admitted to the Hospital for study of behavior problems arising from social maladjustment at home or in school. Two cases of hysteria were also included. Pertinent details regarding the histories and results of other examinations are briefly summarized in connection with the presentation of the experimental data. The general neurological examinations, spinal fluid findings and x-ray plates of the skull were essentially normal and the Wassermann and tuberculin tests were negative in all of the cases presented here. Encephalographic studies after replacement of the cerebrospinal fluid by air (Dandy 6) were not included in this series of cases. However, a fairly large number of encephalograms were made subsequently in similar cases according to the modified technique outlined by Pancoast and Fay (7) and by Pendergrass (8). These have contributed little to our study since the pictures in the mildly epileptic children were practically indistinguishable from those of the non-epileptic subjects. The amount of removable cerebrospinal fluid was likewise essentially the same in the two groups.

The spinal fluid pressure was determined regularly after the first seizure in the epileptic patients and at the height of the reaction in six of the non-epileptic subjects studied. With the child lying quietly in the horizontal position on one side with the head slightly extended, water manometer readings were made before any fluid had been re-

moved. Readings were obtained between two seizures in several cases.

All patients were kept in bed under close observation, either in the special metabolism ward, where complete study of the water and mineral balances could be conducted, or on the regular pediatric divisions of the Hospitals at the University of Rochester or at the University of Minnesota. The mixed diet ordinarily used on the pediatric services was given at first but later this was replaced by diets comparatively low in NaCl. Two of the epileptic patients were kept on basic diets, consisting exclusively of heavy cream, sugar and water the purpose being to limit the mineral intake to an extremely low level, except for short periods when carefully weighed amounts of NaCl were added for a special purpose.

While under observation the patients were weighed regularly every 6 or 12 hours on scales sensitive to 5 grams. The bladder was emptied just before the patient was weighed. For each subject, the 24 hour diet as well as the water was divided into four equal amounts, which were given at 6 hour intervals immediately after the patient had been weighed.

To produce a positive water balance, water was given at the rate of from 2 to 5 cc per kilogram of body weight per hour at either 3 or 4 hour intervals simultaneously with a sufficient amount of the anti-diuretic extract from the posterior lobe of the hypophysis (either pituitrin or pitressin P.D.) to prevent water diuresis. In the earlier experiments the extract was administered intranasally on a pledge of absorbent cotton, but the subcutaneous route was later adopted because it gave less variable results. The water and the extract were discontinued only when the patient had a seizure or manifested fairly marked symptoms of overdosage, such as abdominal cramps, excessive vomiting or slight headache. The body weight usually increased by 2 per cent or more before these symptoms appeared.

For the experiments presented in Charts 6 and 7, the patients were kept in the metabolism ward under the constant supervision of special nurses. The water intake per day was the same throughout the period of observation, and that for each day was divided into 8 equal portions given at 3 hour intervals simultaneously with the administration of pitressin. The diets, consisting of cane sugar and 40 per cent cream in non ketogenic proportions, were calculated to meet the patient

energy requirements under ordinary conditions. Because of the shortness of the experimental period, it was thought unnecessary to give special attention to the protein or vitamin content of the diet. All food was given during the first two hours of each 6-hour period. The urine was collected quantitatively at the end of each period. The patient was weighed at the beginning of each 6-hour period, after the bladder had been completely emptied and before food or water had been given. The temperature and humidity of the room were such that visible sweating did not occur. The insensible water loss was calculated from the body weight changes.

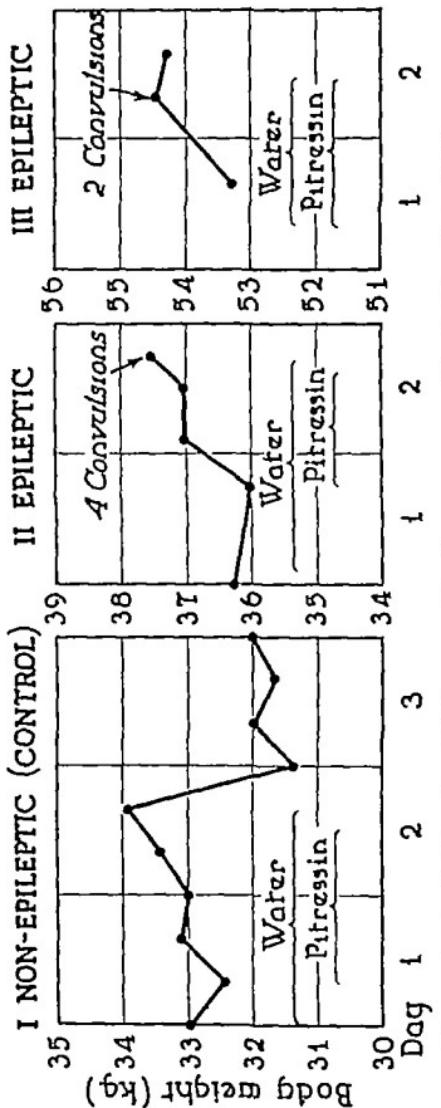
"Water output," as shown in the balance charts, designates the water of the "insensible perspiration" plus that of the urine and feces. "Water intake" includes the drinking water, the preformed water of the food and the calculated water of oxidation. The NaCl, which was given three and one-half days in the first, and four days in the second experiment, was taken in the drinking water in hypotonic concentration (about 0.4 per cent) at a uniform rate. We wish to emphasize the important fact that the salt was at no time given in hypertonic concentration. Full cooperation of the patient and his parents was secured for each study made.

#### RESULTS

For economy of space and for the sake of clearness, the results of representative experiments have been condensed on seven graphic charts, which are practically self-explanatory.

On Chart 1 are presented the data from studies on two patients giving histories typical of mild "essential" epilepsy, and on one non-epileptic control case in the same age period.

The control subject, V. M., was a comparatively healthy girl whose chief complaints were nervousness, enuresis and poor adjustment in school. Thorough examinations for evidence of urological, nervous, metabolic and circulatory disease were negative. She had never had signs of any convulsive disorder. D. M., the first patient, was an Italian boy in the same age and weight group who was referred from another hospital dispensary with the diagnosis of "genuine" epilepsy. He gave a definite history, dating back 3 years, of occasional severe *grand mal* seizures, which usually occurred in the early morning hours.



All received the house diet during the experiments. During the periods indicated by brackets each subject received at 4-hour intervals 500 cc. of water and an intranasal application of 0.5 cc. of pitressin.

and were followed by stuporous sleep. The last attack had been two days prior to his admission to our Hospital. Except for dental caries, his general condition was found to be excellent. The second patient, G B, a somewhat older and larger boy was brought to the Hospital for examination by members of a children's-aid society, who had obtained the history that there had been typical generalized epileptiform convulsions for three years, which had gradually become more frequent. During the previous year the seizures had occurred regularly every three or four weeks, the last having been three days before he entered the Hospital. Although there was slight mental subnormality, his general condition was essentially normal.

It is evident that the antidiuretic effect of the hypophyseal extract, which was administered intranasally, was of the same order in the non-epileptic as in the epileptic subjects. In fact, the percentage weight gain was slightly greater in the first case than in the two latter. Following the gain in weight, convulsions occurred in the epileptic patients as the first prominent effects of the test. An unusual feature of the reaction in the case of D M was that he had four convulsions in quick succession, whereas in no previous attack had he experienced more than one. That the control subject, in whom no convulsive phenomena were provoked, had a sufficient amount of water and extract for a fair test is shown not only by a large gain in weight but by the fact that headache and vomiting occurred as well.

The initial effect of increased water intake before the antidiuretic extract was given, namely, a loss in body weight, was the same in the normal subject as in the epileptic. The excessive loss of weight which followed withdrawal of the antidiuretic effect and water in the control case is regularly observed in both normal and epileptic subjects. This will be discussed later. Unfortunately, the weights of the two epileptic patients were not recorded after the completion of their tests.

The experiments on Chart 2 are presented in part to illustrate the frequently observed variability of the effect on weight of pitressin, when it is administered by surface application within the nostrils. When the water intake is high, as in these cases, diuresis tends to occur from time to time in spite of the antidiuretic, thereby interfering with the object of the test. Subcutaneous administration has given more consistent results than intranasal in our experience.

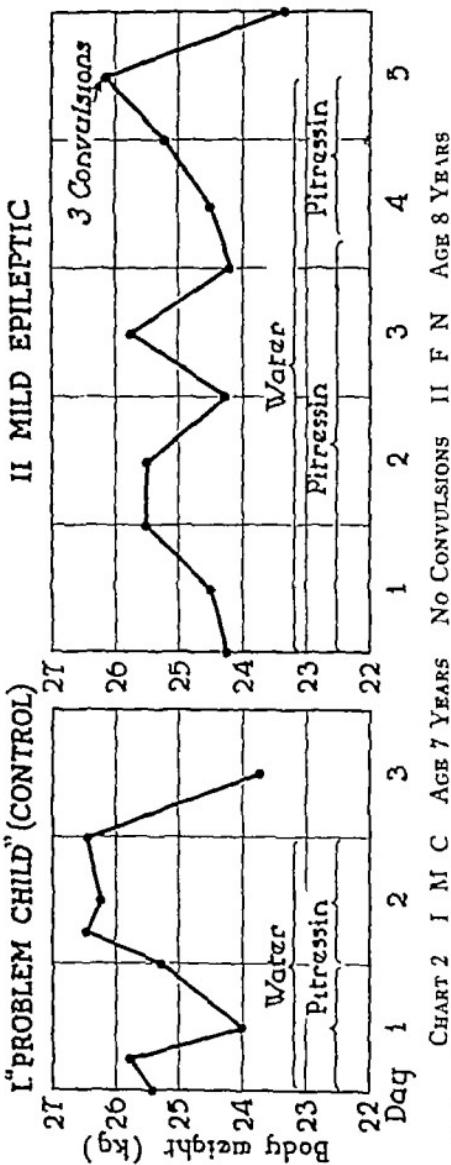


CHART 2 I M C AGE 7 YEARS No CONVULSIONS II F N AGE 8 YEARS  
 Each subject was on the house diet and was given a total of 600 cc. of water every 4 hours over period indicated by brackets. Pitressin was first administered intranasally (0.5 cc every 4 hours) as indicated by first brackets but later subcutaneously (0.3 cc. every 4 hours) as indicated by second brackets

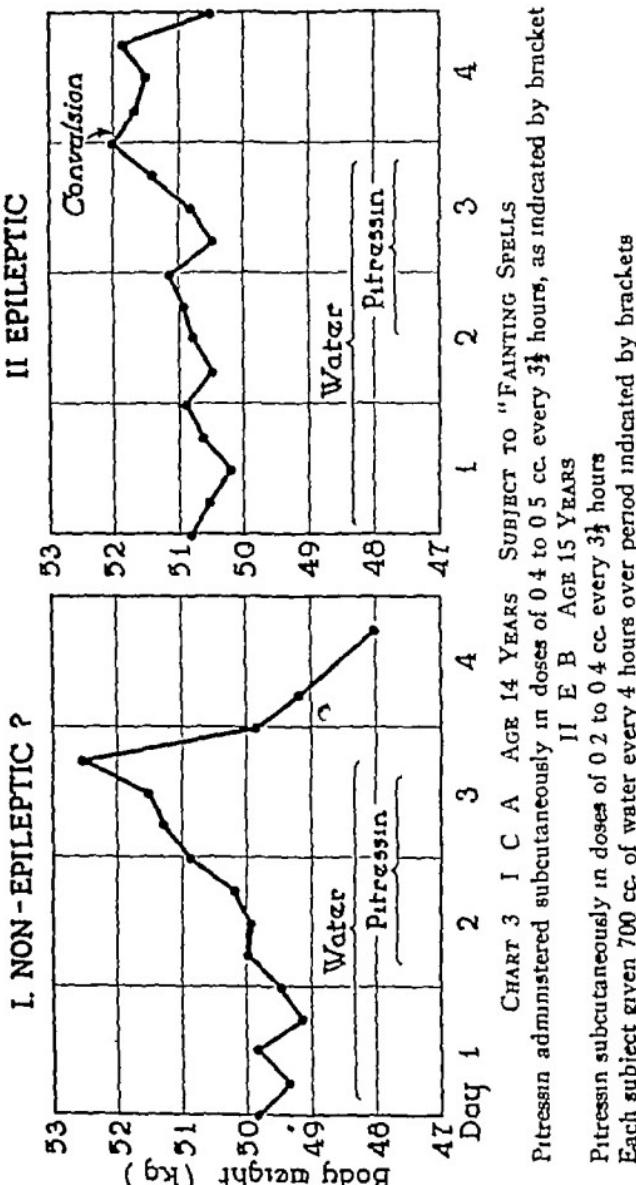
This chart again shows that the establishment of a positive water balance, indicated by the sudden gain in body weight, induced convulsions in the epileptic, but not in the non-epileptic subject. The maximum fluctuations in weight were approximately the same in both.

In connection with this particular experiment, the results on another patient receiving pitressin intranasally should be mentioned because of his unusual reaction. The subject was a boy 15 years of age, who gave a typical history of *grand mal* epilepsy since the age of 9 years with infrequently occurring seizures. The test was performed as in the present experiment. The feature of special interest in this case was that after the storage of water had reached a level at which a seizure might have been expected, the patient experienced a definite aura without having a true seizure. Immediately after this reaction, diuresis developed and did not disappear so long as water was forced, in spite of continued intranasal application of the antidiuretic extract. We have not been able to explain this anomalous physiological reaction, although we have often seen polyuria follow a completed epileptic convolution when no antidiuretic extract was being given.

Chart 3 presents data from tests on two patients, both with the tentative admission diagnosis of "epilepsy."

C A gave an indefinite history of "nervousness" and "fainting spells," which had occurred at infrequent intervals. She usually complained of "feeling faint" before swooning. She had at times sunk to the floor unconscious, but had never injured herself. This had happened when she was in an over-heated room and again when dancing. Frothing at the mouth, biting of the tongue, loss of sphincter control, violent muscular contractions or prolonged periods of drowsiness or sleep after attacks never occurred. She was somewhat retarded mentally, but appeared normal otherwise. It was the impression of Dr. Ferguson of the Neuropsychiatric department, who saw her in consultation before the test was made, that she was not a genuine epileptic.

E B, on the other hand, gave a definite history of convulsions, the first of which had appeared at the age of 9 years. They had recurred biennially up to the age of 13 years, when her menstrual periods began. Since that time, she had had typical *grand mal* attacks every few months. Her mental development has remained essentially normal, however.

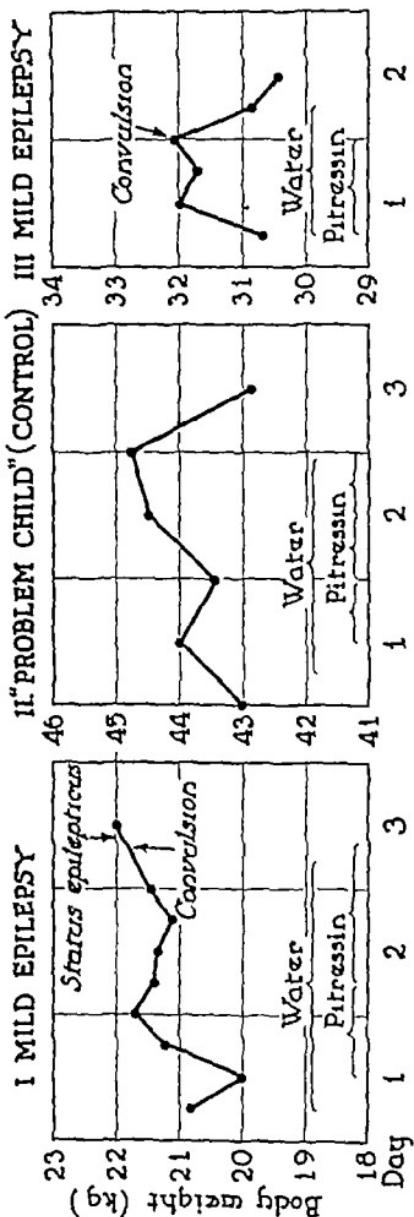


The difference in reaction of these two girls to the positive water balance test was very striking. The experimental conditions were essentially the same in both cases except for the fact that E B was given somewhat smaller doses of pitressin. Although the conditions of the test were thus made much more extreme in the case of C A , finally producing headache, vomiting and a fairly marked vasomotor response she had no sign of fainting or of a convulsive seizure. On the other hand, E B , with but half the gain in weight from water retention and with very little evidence of the other symptoms mentioned, had a typical *grand mal* attack preceded by a definite aura. Following withdrawal of the pitressin, E B showed delay in diuresis not seen in any other patient.

The three experiments presented on Chart 4 are of special interest because each of the patients involved exhibited a peculiar diagnostic problem requiring more information for its solution than that available from the ordinary neurological examination. Subsequent observation has confirmed the accuracy of the conclusions indicated by the tests.

M J B gave a history suggestive of possible brain injury at birth, although at the time of our examination there were no neurological signs of residual organic disease and no evidences of mental retardation. The mother's labor was said to have been precipitous. The patient had one generalized convulsion during the first week of life and at that time red blood cells were found in the spinal fluid. The next convolution occurred three years later. This was followed by hemiparesis which did not disappear entirely for several days. No seizures were seen thereafter for two years. At the end of this period, two weeks before admission to the Hospital, however, a third severe generalized convolution occurred, which could not be explained on any other basis than that of epilepsy. There was a family history strongly suggestive of this disorder.

Before the performance of this special test was considered, we were requested to give an opinion regarding the cause of her convulsions. She had then been in the Hospital for over a week for careful neurological and psychiatric study, the results of which were essentially negative, and was about to be discharged with the diagnosis, "nervous child." We were reluctant to perform the test because of the history of paresis



House diet 300 cc. drinking water every 4 hours Pitressin 0.5 cc intranasally every 20 hours and 0.2 to 0.3 cc. subcutaneously thereafter for period indicated by second bracket

II E M C AGE 13 YEARS

No convulsion Diet Protein 43, fat 64 and carbohydrate 258 grams Total water intake 670 cc every 4 hours Pitressin 0.5 cc. intranasally every 3 hours for 3 doses then 0.4 cc and 0.5 cc. subcutaneously every 3½ hours for 3 doses each

Diet Protein 31 fat 45 and carbohydrate 180 grams. Water intake 600 cc every 4 hours Pitressin 0.3 cc subcutaneously every 3½ hours

following one of her previous attacks. However, the parents and the family physician were willing to assume responsibility for possible ill effects from it because they were somewhat dissatisfied with the indefiniteness of the provisional diagnosis.

During the first 24 hours the test was without definite effect because of over-caution regarding the dosage of pitressin. When the latter was increased, however, seizures resulted within 18 hours. It is probable that an unobserved seizure occurred just before the last dose of the extract was given because the patient had otherwise unexplained enuresis and drowsiness. When the attacks began to appear with great frequency, they were brought under control by the induction of light chloroform anesthesia. No noticeable ill effects resulted. This particular experience, however, emphasizes the desirability of terminating the period of antidiuresis as soon as the first seizure has occurred. Since her discharge from the Hospital, the diagnosis of epilepsy has been further confirmed by recurrence of seizures on two occasions several months apart.

As shown in the second division of the chart, no convulsion was induced when the test was performed on a "behavior-problem" child, who gave a history of six or seven atypical "fainting spells" during the previous three years. In none of these had there been observed complete loss of consciousness, convulsive movements, frothing at the mouth, cyanosis or injury from falling. Members of the staff in Neuropsychiatry had never considered her to be an epileptic. Her "spells" were regarded as a feature of her behavior disorder. As shown on the chart, no seizures occurred in spite of a gain of two kilograms in body weight from water storage. Vomiting, abdominal discomfort and headache finally necessitated the termination of the test. That this patient served as a reliable control is confirmed by her subsequent history.

From the clinical point of view the patient, who served as subject for the experiment presented in the third division of Chart 4, was in several respects the most interesting studied. While she gave a long history of *grand mal* seizures in every respect typical of true epilepsy, neurological examination revealed the absence of a normal gag reflex and the presence of peripheral anesthesia with the "stocking and glove" distribution characteristic of hysteria. Further investigation of the history

brought out the fact that during the previous two years the patient had suffered from "nervousness," and transient attacks of dizziness, headache and precordial pains, with no physical findings to explain such symptoms. The latter appeared to have no relationship to the seizures, but had developed subsequently, during a period when the mother was suffering from severe exophthalmic goiter which kept the family as well as herself under "high nervous tension." The child's symptoms, like the mother's, had greatly improved following removal of the mother's goiter. Until these features suggesting hysteria were discovered, the clinical diagnosis, based upon the character of the convulsive seizures, had been epilepsy.

When the antidiuresis test was performed, every precaution was taken to keep the patient from knowing its purpose or from knowing that she was being observed. That the convulsion which followed was not hysterical in character is practically certain. It occurred only after the patient's body weight had been increased over 4 per cent by retained water. As described by Dr. Clausen and members of his staff, who were making ward rounds in an adjoining room when called to see the patient, the convulsion was typical of the *grand mal* seizure of genuine epilepsy. A characteristic aura was reported by the supervising nurse. The pupils were widely dilated and the eyeballs were turned upward and to one side. There was a definite tonic phase with cyanosis and twisting of the body to one side, followed by a clonic phase with drooling and frothing at the mouth. There was no definite loss of sphincter control and the tongue was not bitten, but the seizure was followed by a long sleep.

The conclusion was drawn that the patient was a true epileptic, with superimposed hysteria of a mild grade, which had manifested itself in other ways than by means of convulsions.

The experiment presented in Chart 5 differed from those preceding in several ways. The patient was an intelligent, well developed, 16 year-old Armenian girl who gave a typical history of so-called genuine epilepsy of one year's duration. *Grand mal* seizures had occurred monthly or bimonthly almost from the beginning. The time selected for the test, as in the previous cases, was within the period when no regular seizure was expected. A preliminary test, with the pitressin administered intranasally, was unsuccessful as regards the physiological response and the production of a seizure.

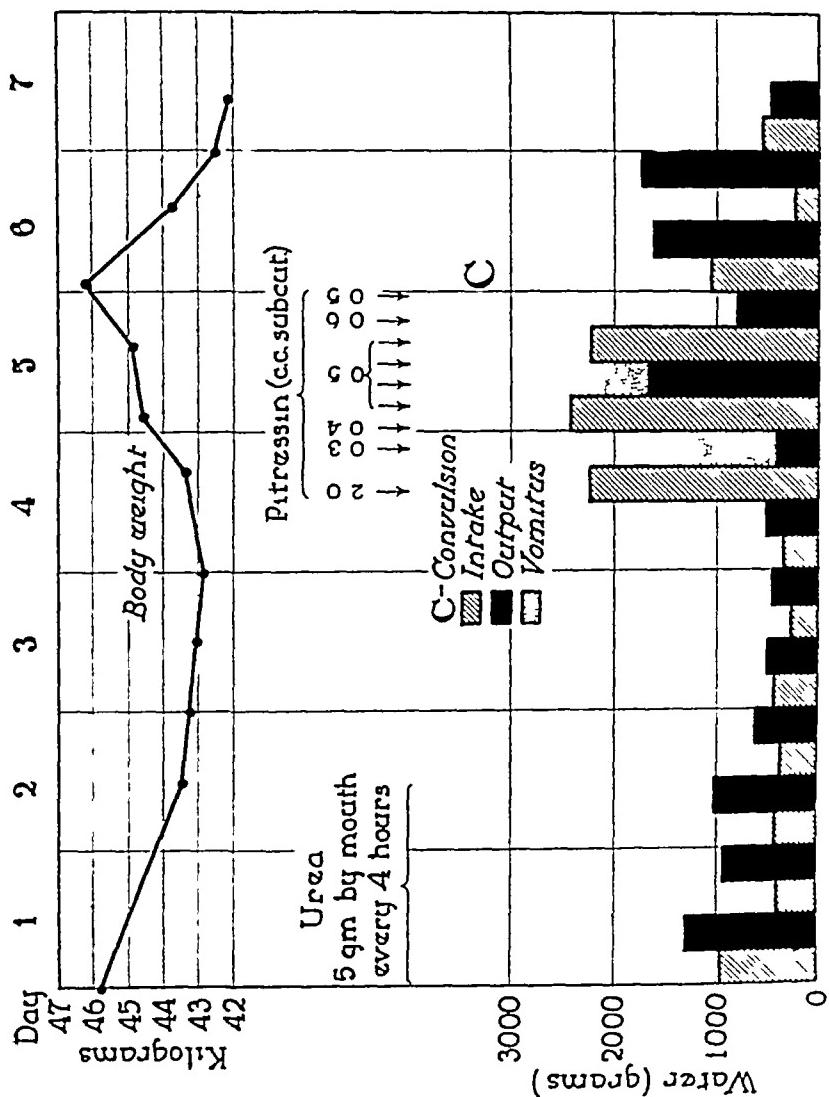


CHART 5 S D AGE 16 YEARS MILD EPILEPSY  
 Diet for 24 hours consisted of protein 75, fat 75 and carbohydrate 175 grams test preceded by period of dehydration

Since the history was so clearly one of epilepsy, it was decided to repeat the study under conditions in which the complete water balance of the body could be accurately determined. A preliminary period of dehydration was included because it had been observed repeatedly in severe epileptics that seizures were likely to be more frequent and more severe on resuming a high water intake following a period of freedom and low intake. It was considered that this might prove to be an additional refinement in the test, to bring out the latent convulsive tendency in the epileptic subject. A considerable degree of dehydration was produced by administration of urea over a period of 36 hours and limitation of the total water intake over a period of three and one half days. Sufficient water was then given over a period of a few hours to completely satisfy the patient's desire for it (over 1000 cc.), after which the routine administration of the antidiuretic extract of the pituitary gland and water was begun.

In order to avoid unpleasant reactions, and to prevent complications such as vomiting, the plan previously evolved was to begin with a small dose of pitressin and gradually increase this until the optimum dosage was reached. Inadvertently, the substitute nurse in charge injected ten times the intended amount of pitressin for the first dose. The patient's reaction to this overdosage was extremely intense. There were marked and persistent pallor of the skin and mucous membrane, with feeling of faintness and collapse, weakness and irregularity of the pulse, abdominal discomfort, dizziness, headache and persistent vomiting but no convulsion nor loss of consciousness. Incidentally this episode, while distressing in one respect, proved to be of great value in that it demonstrated beyond reasonable doubt that the convulsions induced in the epileptic subjects studied are not associated primarily with the vasopressor action of the extract, but with its antidiuretic or hydrating effect.

After the pronounced symptoms of pitressin overdosage had subsided, the test was continued according to the original plan. A typical *grand mal* seizure occurred about 36 hours later, after nearly three liters of water had again been stored in the body. During the 24 hours preceding the convulsion, no vomiting, no headache and no striking vaso pressor effect were observed. Spinal fluid pressure taken shortly after the convulsion was 125 mm. of water.

The marked loss in body weight after the termination of the test is of special interest. It was obviously due to a massive loss of water, largely by way of the kidneys. It is somewhat surprising that 30 hours after the convulsion the weight had fallen below the minimum for the dehydration period. Except for the period of about 20 hours when there was vomiting from overdosage of pitressin, the diet, containing 1700 Calories, was apparently well taken. This effect on the body weight, which is observed in the controls as well as in the epileptic subjects on the withdrawal of the antidiuretic agent, is similar to that of simple water diuresis, but is more marked. Whether or not this is due solely to previous loss of minerals, so that the storage depots are temporarily unable to retain their usual amount of water, or in part to other factors, is uncertain.

The purpose of the following two experiments, which were conducted in the metabolism ward under rigidly controlled conditions, was to throw some light, if possible, upon the *modus operandi* of the water and pituitary extract in provoking seizures in epileptic subjects. It is obvious that the addition of a large volume of distilled water to the extracellular body fluids must result in dilution of these solutions bathing the cells of the body. Under ordinary conditions, the drinking of similar amounts of water results in but little dilution, because the extra water is excreted very promptly. To the extent that minerals are carried from the body with it, there will be loss of extracellular body water also to maintain the normal concentrations.

When, however, the increased water intake is accompanied by administration at frequent intervals of an efficient antidiuretic agent, the normal mechanism for adjustment cannot operate successfully and dilution of the fluid about the cells is inevitable. If this condition persists for a sufficiently long period of time, it is practically certain that the cells will take up extra water because of the diminished osmotic pressure of the fluids surrounding them. It may be presumed that this dilution of the cell contents with resulting disturbances in the ionic equilibria results in the increased irritability of nerve tissue eventuating in the convulsion. It was thought desirable, therefore, to study the problem in a way such that the importance of the dilution factor might be more accurately appraised.

In the limited time available for this study, it was planned to keep two mildly epileptic children, who were known with certainty from

previous observation to have *grand mal* attacks at relatively infrequent intervals, on basic diets extremely low in NaCl and on uniform water intake at a rate between 3 and 5 grams per kilogram of body weight per hour. After preliminary periods of 36 hours, the antidiuretic principle was to be given at 3 hour intervals until seizures were produced. After an interval of 24-hours, which should be allowed for recovery from the effects of this procedure NaCl in sufficient amount to make approximately a 0.6 per cent solution of the fluid estimated to be retained, was to be given over a period of 24 hours. This was then to be continued and at the same time the antidiuretic agent was to be administered as before until a seizure should occur, or until other indications for its discontinuance should appear, after which the salt would be continued for an additional 24 hours. The remainder of the plan was to repeat the first part of the experiment 24 to 36 hours after discontinuance of the NaCl, as an added check. The diets and water intake remained the same throughout the entire period.

As shown in Chart 6 the experiment was carried out essentially as planned. The important feature of the results obtained was that a much longer time and a much greater gain in weight were required for the induction of a seizure when NaCl was given than when it was not given. The convulsion, which occurred in the foreperiod followed the third dose of pitressin that in the sodium chloride period came approximately three hours after the 12th dose, and that in the last period after the 6th dose. Before the seizure of the sodium chloride period occurred the body weight reached a peak more than one kilogram above that of the low mineral-diet period.

In the next experiment (Chart 7) there was a slight deviation from the original plan in that no pitressin was administered during the fore period. In this instance during the preliminary period of increased water intake, instead of the usual loss of body weight due to water diuresis, there was a net gain of 2 per cent due to water retention and at the end of 12 hours the patient had a severe convulsion. The usual water diuresis then followed during the next 24 hours. The increase in weight during the succeeding 24 hours due alone to the addition of NaCl, while materially greater than the first gain, was unaccompanied by a seizure. Nor was there a seizure when the body weight was made to increase by more than 5 per cent by the simultaneous administration

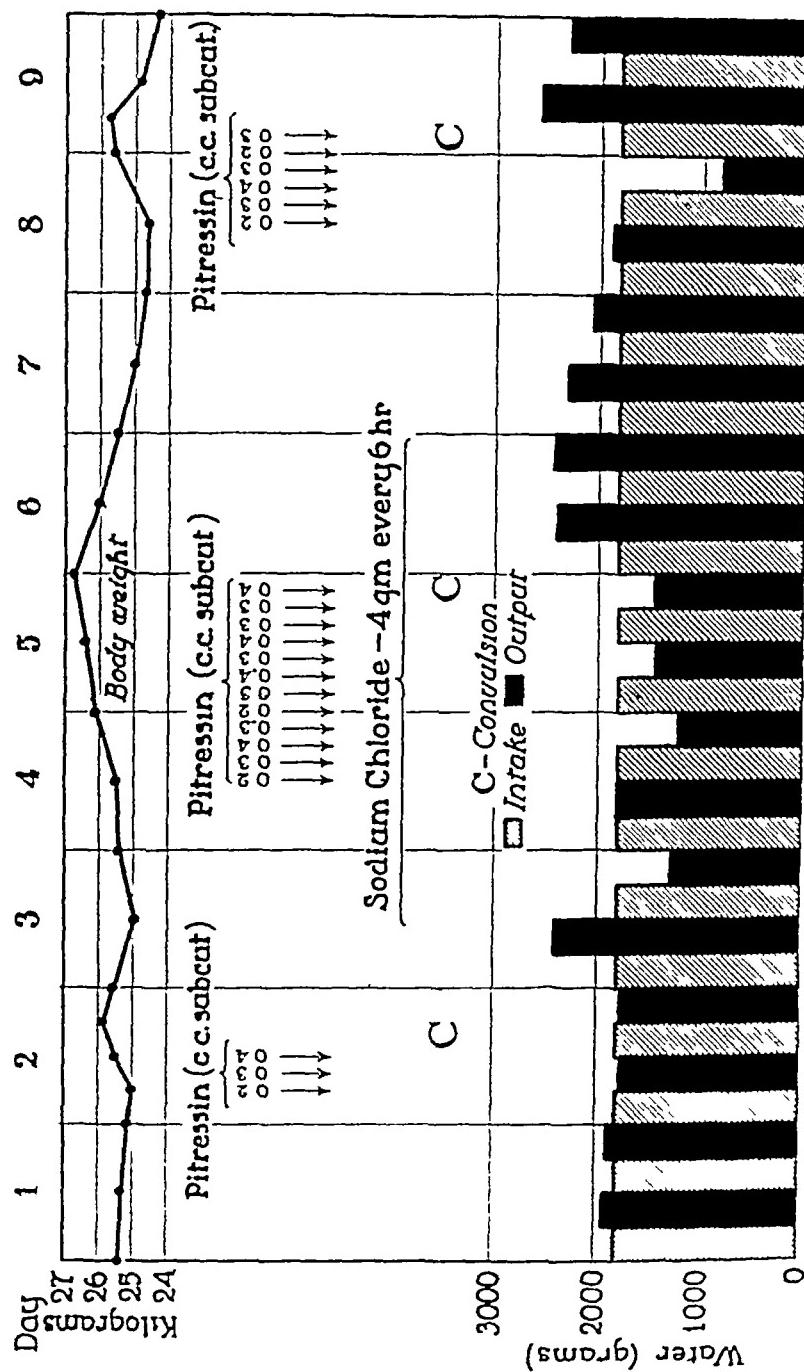


CHART 6 N Γ ACF 10 YRS Mild Epilepsy  
Diet for 24 hours consisted of 160 grams of 10 per cent cream and 240 grams of cane sugar

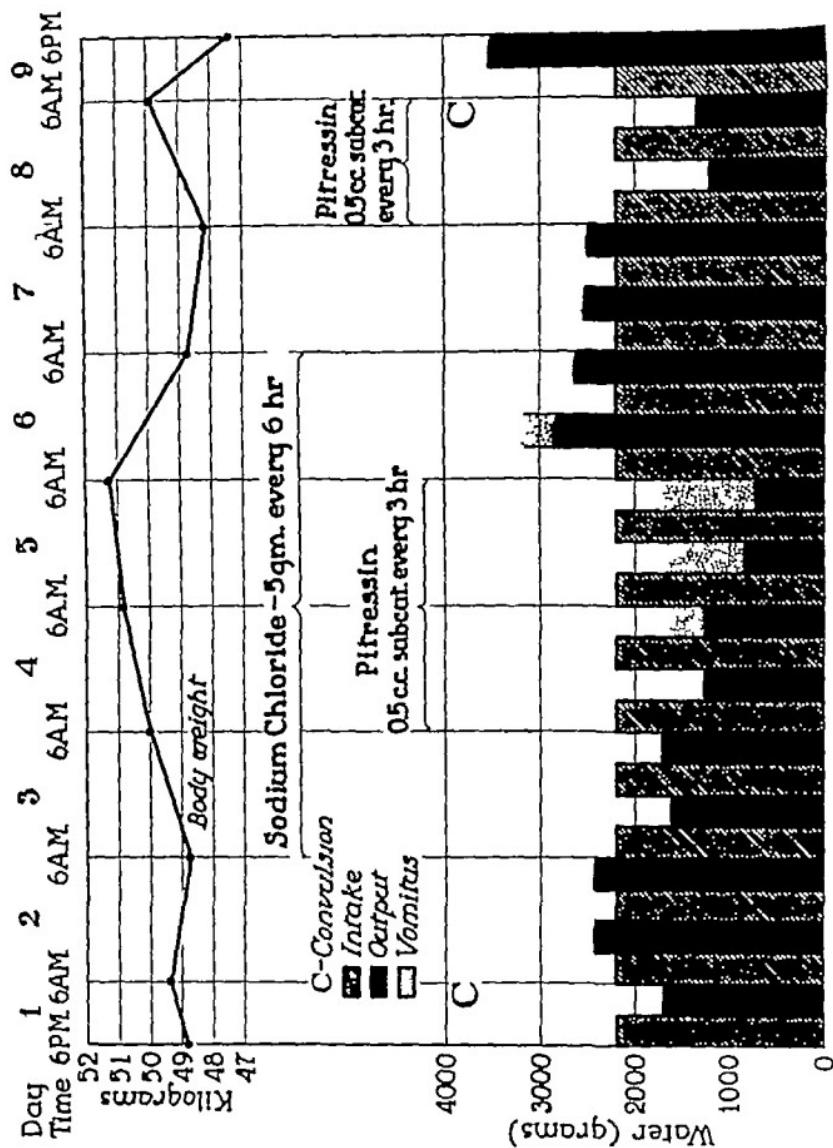


CHART 7 E. B. AGE 14 YEARS MILD EMULSION  
Diet for 24 hours consisted of 180 grams of 40 per cent cream and 320 grams of cane sugar

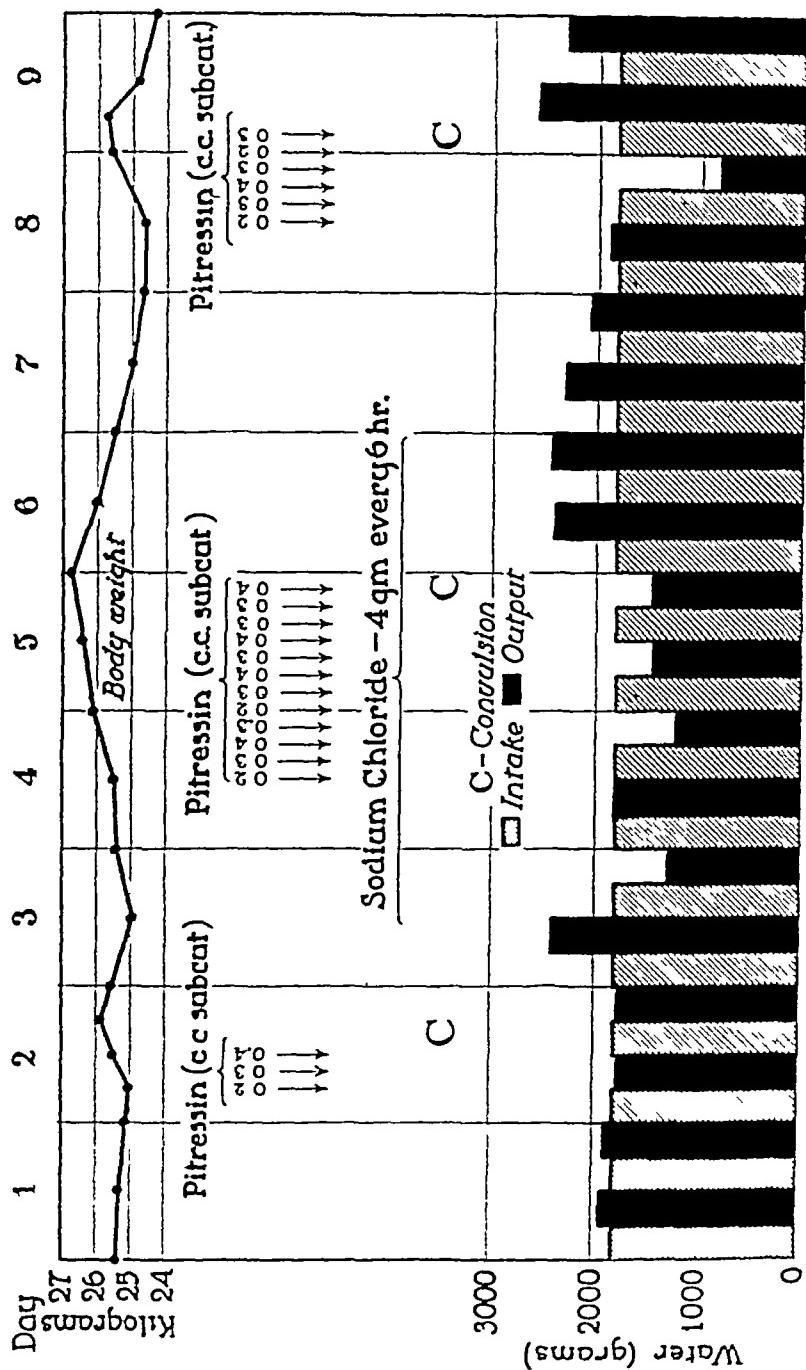


Chart 6 N Γ AGE 10 YEARS MILD EPILEPSY  
Diet for 24 hours consisted of 160 grams of 10 per cent cream and 240 grams of cane sugar

water balance test was performed on him at the request of the staff in Neuropsychiatry during one of his "free" periods. No type of seizure resulted from prolonged continuation of the procedure, although he had headache, vomiting, vasomotor symptoms and a gain in body weight of more than 5 per cent. Some days later, after the effects of the test had entirely disappeared, he was made to have one of his ordinary hysterical "convulsive attacks" by a clever arrangement of circumstances discussion of which is beyond the scope of the present paper. Details of this case will be reported independently by Dr C. B. Horton.

#### DISCUSSION

It is evident from the foregoing results that typical *grand mal* seizures can be induced in children with epilepsy, even in the early stages and during the free intervals between spontaneous attacks, by materially increasing the water intake and administering at the same time a sufficient amount of antidiuretic pituitary extract to prevent water diuresis. A low mineral intake during the test apparently favors this type of response, whereas preventing dilution of the body fluids tends to interfere with the induction of seizures. In essentially normal children, presenting simple problems of social maladjustment or ordinary "nervousness," and in other non-epileptic subjects with hysteria or with typical "shaking" or "fainting spells" the procedure when applied in the way described here does not cause convulsive reactions of any kind. Apparently very little, if any harm to the patient results from it. We believe, therefore, that it may be employed as an aid in differential diagnosis in early or obscure cases, in which other more simple methods of provoking the characteristic seizure fail.

In addition to their practical implications, the foregoing experimental data appear to us to contribute significant information on what may be termed the physicochemical pathology of "genuine" epilepsy. Before our tentative interpretation in this connection is presented, however, other possible explanations of the results obtained will be briefly discussed.

Because of the tendency for convulsions to follow interference with blood supply to the brain (10) and because anoxemia has been shown by Lennox and Cobb (4) to favor the occurrence of seizures in epilepsy, it was natural at first to think that the seizures might be due to the vaso-

of hypotonic salt solution and pituitary extract, although headache, vomiting and circulatory symptoms were so severe that the experiment had to be discontinued on the fourth day. Evidently, the prevention of dilution of the extracellular body fluids in this experiment interfered with the induction of a seizure. The third phase of the experiment was carried out 24 hours after withdrawal of the NaCl. In spite of the fact that not all of the NaCl given had yet been excreted, as shown by subsequent measurements, repetition of the antidiuresis test, without addition of NaCl, resulted in a typical *grand mal* attack within 20 hours. It is planned to compare the effects of other ions with those of Na and Cl in experiments similar to these. In a study on the relative effectiveness of the various ions in inhibiting the swelling of brain tissue, placed in distilled water, Haldi and his coworkers (9) found a considerable difference between the different ions. The bivalent were more efficient than the monovalent ions in preventing swelling.

Since the experiments described here were done, we have seen the antidiuresis test carried out on more than forty mildly epileptic children, in all but one of whom seizures have resulted as in these cases. None of the eighteen definitely non-epileptic subjects observed have reacted in this way, although in the majority of them the duration of the test has been longer, the weight increase greater, and the other symptoms of overdosage more marked. In but one case so far have we seen genuine *petit mal* seizures definitely induced by this procedure.<sup>3</sup> In this particular patient the minor seizures were provoked on three successive occasions. On the first of these, when larger doses of pitressin were given, she had one *grand mal* attack, as well as several *petit mal* seizures. We have watched the effect of the test on but one patient who had frequent spontaneous *petit mal* attacks without ever having had *grand mal*. In this instance the special nurse reported an increase in the frequency and severity of the *petit mal* seizures. This particular problem undoubtedly deserves further investigation, because the mechanism of *petit mal* seems to differ in some respects from that of *grand mal* as seen in the mild cases described in this paper.

One of the other cases, which was of special interest to us, was that of a man 23 years of age with typical hysterical attacks. Our positive

<sup>3</sup> To be reported in connection with mineral and water metabolism studies being made by McQuarrie, Ziegler and Engel.

convulsion in each case was above 200 mm of water, the values recorded immediately before and a few minutes after it were well within normal limits. No difference has been found between the pressures of the epileptic patients and those of the non-epileptic subjects, on whom determinations have been made under the same conditions. The rate of water administration to our patients was but one twentieth to one thirtieth that used by Rountree to produce convulsions in his normal animals. Unless an unexpectedly large fraction of the water retained in the body were stored within the skull, it is most unlikely that sufficient increase in general pressure to cause seizures should develop from the comparatively small amount of water given. We have been unable to influence the occurrence of convulsions in our subjects by increasing the intracranial pressure by jugular compression. Even in other convulsive conditions, in which increased intracranial pressure is conceded to be a contributing causal factor, the exact nature of the pressure effect and its relation to the convolution are not known. It is conceivable that the injury to the brain cells from direct pressure and from interference with blood supply may be sufficient to alter the permeability of their membranes to water and electrolytes.

In Rountree's normal animals with water intoxication there was observed no tendency for water to localize in the subarachnoid spaces in spite of the enormous quantity administered (13). In the few epileptic cases in which attempts have been made by Thompson to remove the fluid from the subarachnoid system quantitatively and replace it by air, first some weeks before, then during and again some weeks after the antidiuresis test, the results have indicated that the total volume of fluid tends to be slightly diminished rather than increased during the time when the water retention in the body is at its maximum.<sup>4</sup> Barbour and his coworkers (14) found that the water content of the brain tissue itself is increased in dogs under conditions similar to those of our experiments. It appears probable to us, therefore, that the volume of cerebrospinal fluid may even decrease as the volume of the brain tissue increases, in keeping with the space-compensation theory of Dandy (15).

We do not regard ordinary intercellular brain edema, which is referred to by some authors, as being of great importance in the causation of epileptic seizures. However, entrance of water or hypotonic NaCl

<sup>4</sup> Personal communication

pressor effect of the pituitary extract. However, three objections to this interpretation have appeared. The first of these is that there has been no regular time relationship between the occurrence of seizures and the transient vasopressor effect of the extract, which follows within a few minutes after the injection. The next is that seizures tend to occur only after a positive water balance of a considerable magnitude has been established. In the third place, while the vasopressor reaction may well be a contributing factor in some instances, the experiment presented on Chart 5 would tend to convince the most enthusiastic proponent of the vascular-spasm theory that it is not of primary importance under the conditions of this test. Were the theory applicable under these circumstances, it would seem certain that the patient should have had a seizure, or more likely many seizures, when she was inadvertently given ten times the intended dose with the production of a vasopressor reaction of almost maximal intensity.

The next possibility which presents itself as the direct cause of the convulsions is increased intracranial pressure, which has been thought by some writers to be an important factor in epilepsy. This was at first considered by Rowntree and his coworkers (11) (12) to be the major factor in the causation of convulsions in experimental "water intoxication," a reaction similar in certain respects to that of our experiments. Generalized convulsions, which were said in their earlier papers to be epileptiform in type, were produced in normal experimental animals by these observers, when water was administered at the enormous rate of 100 cc per kilogram of body weight per hour or when half of this amount of water was given in conjunction with sufficient pituitrin to prevent diuresis. In a later paper Rowntree (13) described the convulsions as resembling those of strychnine poisoning and expressed the belief that a disturbance in the balance of the electrolytes is of paramount importance in their causation.

The chief objection to the theory of increased intracranial pressure in connection with our experiments is that the spinal fluid pressure readings, taken regularly on the patients soon after their seizures and in a number of instances just before, have varied between 80 and 130 millimeters of water, which is well within the normal range of variation. In two instances convulsions occurred while the spinal fluid pressure was being measured. Although the reading taken at the height of the

2 It is shown that typical *grand mal* seizures can be induced within 12 to 48 hours in the epileptic, but not in the non-epileptic subject, by giving water at the rate of from 2 to 5 cc per kilogram of body weight per hour while maintaining effective pituitary antidiuresis.

3 This difference in response between epileptic and non-epileptic patients may be helpful in establishing the diagnosis early in an obscure case.

4 Under the conditions of the experiments described here, dilution of the extracellular body fluids appears to be an essential factor in the induction of seizures since administration of an amount of NaCl just sufficient to prevent this tends to interfere with their occurrence.

5 The results presented lend strong support to the view that the mechanism for controlling semipermeability of the brain cell membranes is inherently defective in the epileptic patient.

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solution into the cells in excessive quantities with resulting dilution of the cell contents and alteration of the normal relationships of both colloids and electrolytes would be considered of very great significance. Some indirect evidence that such a translocation of water may precede the occurrence of a seizure provoked by sustained pituitary antidiuresis has recently been obtained from a combined study of the water and mineral balances in a severely epileptic patient (16). The view was expressed in an earlier paper (2) that the beneficial effect of the dehydrating regimen used in the treatment of epilepsy might depend in large part upon its preventing overhydration of the brain cells, rather than upon its decreasing the quantity of fluid in the subarachnoid spaces, as maintained by Fay (17). The results from the present study, as well as certain other experimental data now available, tend to support that position.

Under the conditions of the experiments reported here, in which the subject on a low mineral diet is forced by sustained antidiuresis to retain a volume of water greater in some instances than that of the total plasma volume, a significant decrease in the total osmolar concentration of the electrolytes in the extracellular fluids of the body is found to take place. Due to the resulting alteration in the osmotic pressure of this fluid, there is undoubtedly a strong tendency for the fixed cells of the brain and of other organs to take up water and to lose some of their diffusible elements. The fact, that convulsions are inducible in the epileptic but not in the non-epileptic subject under these particular conditions, suggests that the barrier, normally preventing free passage of water and various solutes into and out of the brain cells, functions inefficiently in the former. This interpretation is consistent with a working hypothesis which has recently been derived from separate studies on the blood lecithin and cholesterol (18) and on the mineral and water exchanges (16) in severely epileptic patients. The central concept in this hypothesis is that an innate deficiency in the mechanism for controlling semi-permeability of the brain cell membranes characterizes the physicochemical pathology of "essential" epilepsy.

#### SUMMARY AND CONCLUSION

1 Experimental and clinical data are presented to show the effects of sustained pituitary antidiuresis and water drinking on mildly epileptic as contrasted with non-epileptic children.

2 It is shown that typical *grand mal* seizures can be induced within 12 to 48 hours in the epileptic, but not in the non-epileptic subject, by giving water at the rate of from 2 to 5 cc per kilogram of body weight per hour while maintaining effective pituitary antidiuresis.

3 This difference in response between epileptic and non-epileptic patients may be helpful in establishing the diagnosis early in an obscure case.

4 Under the conditions of the experiments described here, dilution of the extracellular body fluids appears to be an essential factor in the induction of seizures since administration of an amount of NaCl just sufficient to prevent this tends to interfere with their occurrence.

5 The results presented lend strong support to the view that the mechanism for controlling semipermeability of the brain cell membranes is inherently defective in the epileptic patient.

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## THE PLASMA PROTEINS IN RELATION TO BLOOD HYDRATION VI SERUM PROTEINS IN NEPHRITIC EDEMA

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The total proteins of the serum have been determined 179 times in 21 patients with nephrosis or nephrotic types of nephritis. On 118 occasions in 15 patients albumin and globulin fractions were determined separately.

The methods employed for analysis of the serum have been described elsewhere (1).

### *Nature of clinical material*

Abstracts with the most significant clinical features of each case may be found at the end of the paper. From consideration of these protocols it becomes at once evident that the clinical material is so far from homogenous that the propriety of discussing it under a single head may be questioned. The cases can be divided roughly into three groups. From the standpoint of both etiology and course the first eight patients clearly deserve a diagnosis of glomerular nephritis. With the possible exception of the third case, 61090, the disease was in every instance preceded by sore throat, and was usually ushered in by an acute stage in which hematuria and transitory hypertension and azotemia were noted. In number 61090 the onset was insidious, but the initial hypertension and azotemia and the subsequent course of the disease were quite typical of glomerular nephritis.

Of the next five patients, three (34753 56577 and 72481) were proved at autopsy to have amyloid nephrosis the other two probably had the same condition. It is not at all unlikely that the succeeding two, 50625 and 15544, also had amyloid disease both had active pulmonary tuberculosis.

The last five have been grouped together merely because the etiology is in every case uncertain. Four of them, 73222, 29122, 80351 and 62246, conformed more exactly than any of the other patients in the series to

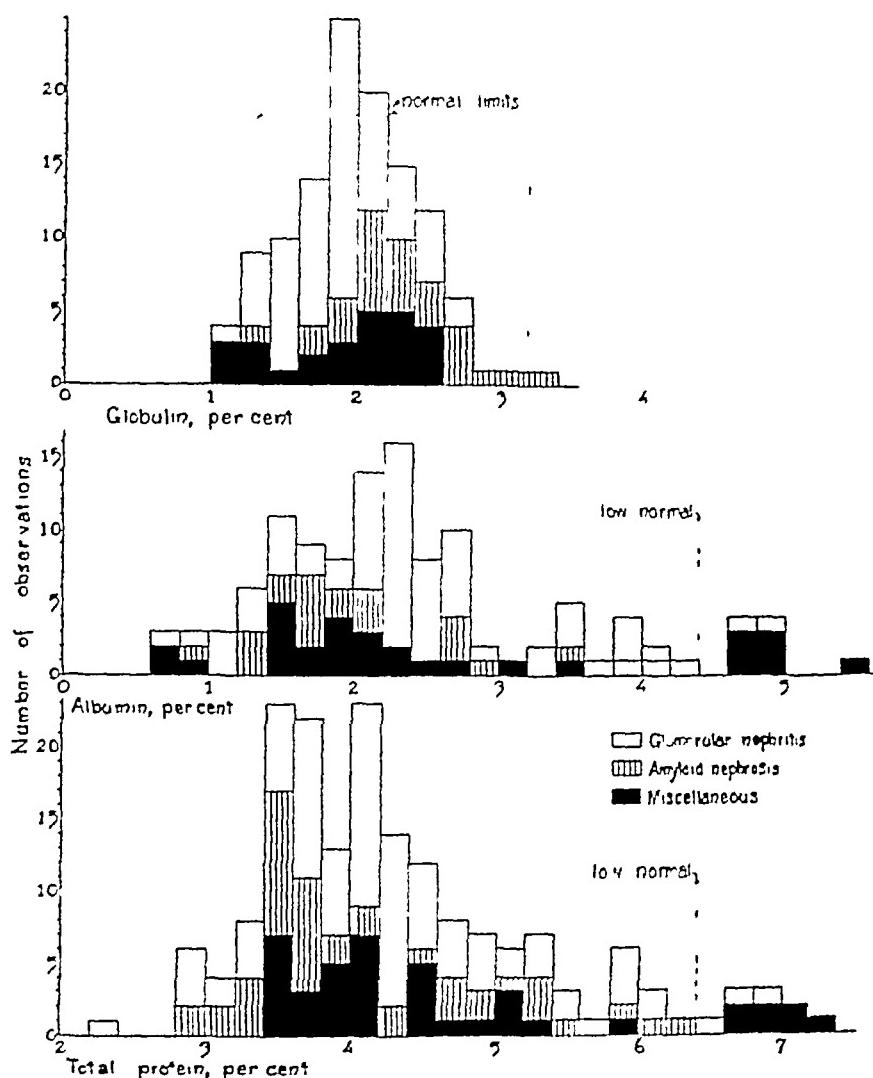


FIG. 1 THE RELATION OF SERUM PROTEINS TO THE TYPE OF NEPHRITIS

Epstein's (2) description of nephrosis. The origin of the disease was insidious, without antecedent infection and unaccompanied by hypertension, azotemia was observed only in 73222 for a short time after admission hematuria was absent or minimal

On the other hand, all the patients, at one time or another, usually over considerable periods, exhibited clinical and functional pictures in distinguishable from that described by Epstein (2) as characteristic of nephrosis, edema massive albuminuria and reduction of serum proteins, without nitrogen retention, high blood pressure, or gross or microscopic hematuria. Basal metabolism and serum cholesterol which were determined in a certain proportion of the cases, served no better to differentiate "pure nephrosis" from the other renal conditions. In another paper (3) it has been demonstrated that the electrolyte abnormalities in the different groups of cases are no more distinctive.

All these facts seem to justify discussion of the functional disturbances in this group as a unit and raise the question whether "nephrosis," as a clinical term, is more than a convenient means of describing a syndrome that may occur in the course of renal diseases originating from a variety of causes.

How impossible it is to differentiate nephrosis from glomerular nephritis and amyloid disease of the kidneys by means of the serum proteins can be seen from Figure 1. Both total protein and albumin may be quite as low in one disease as another. Globulin tends to be somewhat higher in amyloid nephrosis than in the other conditions. This is presumably referable to the chronic infectious process and not to the renal lesion (1, 4). The globulin increases are not great enough or consistent enough to aid in the diagnosis of amyloid disease.

#### *Relation of edema to total serum protein*

The relation of serum proteins to edema in this series is illustrated by means of frequency charts, in Figure 2. The close correlation is at once apparent. Edema was almost invariably present when total proteins were below 4 per cent, while it was observed in only three instances when the proteins were above 5 per cent. The latter exceptions to the general rule, moreover, occurred in two patients, 15544 and 83434, who had in addition to renal disease, distinct signs of heart disease with circulatory failure.

Although it was sometimes possible to induce some degree of diuresis while the proteins were below 4 per cent, complete elimination of edema was effected under these circumstances in only one instance. In this instance serum albumin was relatively high, 2.39 per cent, while globulin was quite low, 1.34 per cent.

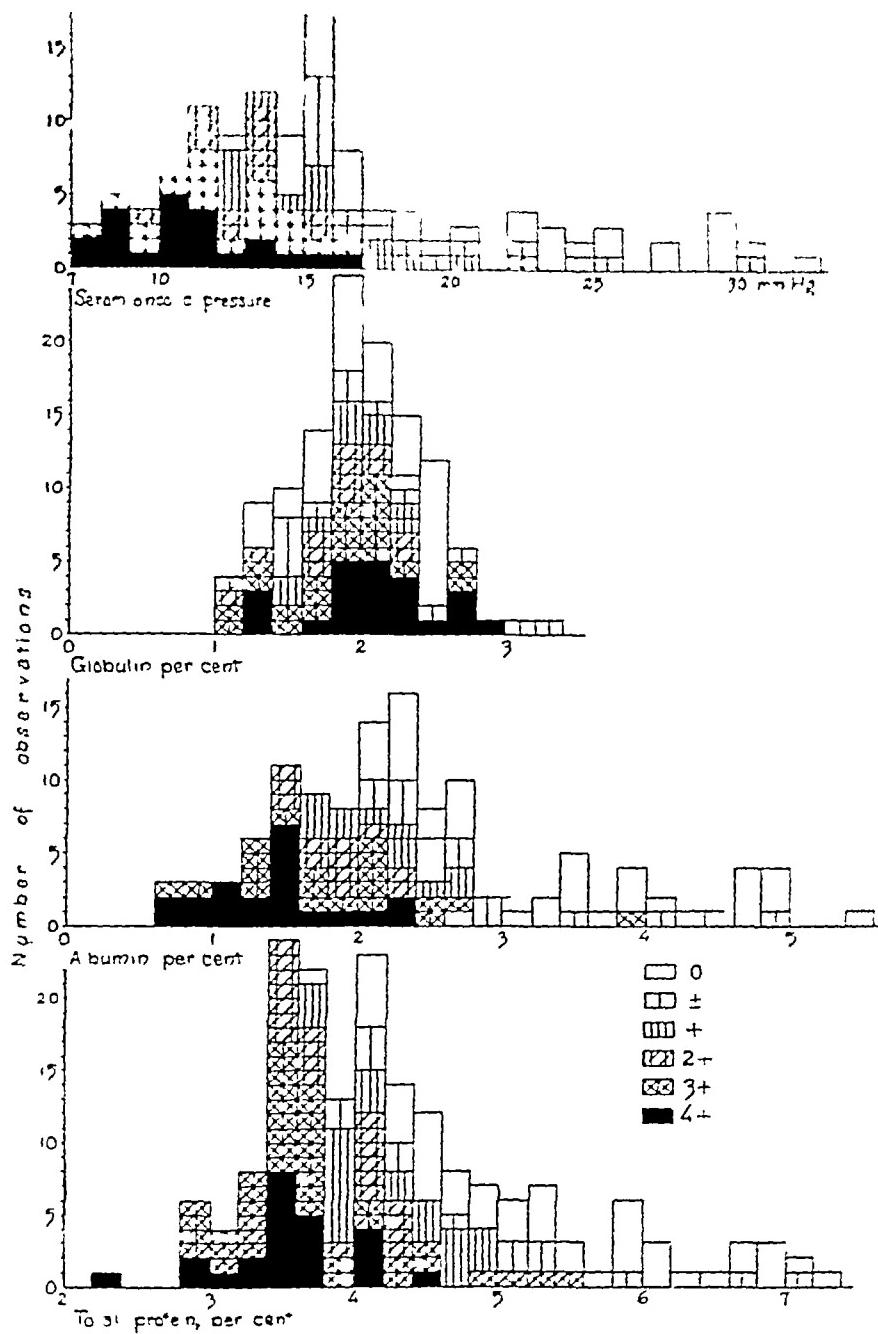


FIG 2 THE RELATION OF EDEMA TO THE SERUM PROTEINS

Estimation of the degree of edema is quite rough.  $\pm$  indicates slight puffiness of the feet noticed by ambulatory patients at the end of the day.  $+$  represents persistent demonstrable swelling of the feet.  $2+$  more extensive subcutaneous edema.  $3+$  subcutaneous edema and serous effusion.  $4+$  extreme general anasarca.

Oncotic pressure was calculated from the serum protein values by the factors of Govaerts ( $5.5 \times$  per cent albumin)  $+ (1.4 \times$  per cent globulin) = oncotic pressure in mm. Hg

With proteins between 4 and 5 per cent edema was sometimes present, sometimes absent. Altogether, demonstrable edema was observed 36 times when the proteins were within this range. Of these 36 observations 20 were made either before treatment was instituted or during periods of diuresis. In these cases either treatment was effectual in eliminating edema or else the serum proteins fell below 4 per cent. Edema persisted in case 83166, in spite of the fact that the proteins rose above 4 per cent, during an intercurrent attack of acute nephritis. In acute nephritis edema does not seem to depend directly on the serum protein level (5, 6, 7). In another instance, case 73222, there was a transitory rise of protein to 4.04 without diuresis during the terminal period of septicemia when the patient had been vomiting continuously. One of the observations was made on case 15544, the patient with heart failure noted above. 8 on case 34854, who proved to have rheumatic heart disease. The 3 remaining instances represent merely transitory rises to between 4.0 and 4.1 per cent.

In this series, therefore, it proved possible in every instance to eliminate edema by treatment when the proteins persistently exceeded 4 per cent, unless there was concomitant cardiac disease with decompensation or a complicating acute nephritis or septicemia. The treatment employed consisted of restriction of salt to 2 grams or less of NaCl daily with little or no restriction of fluids. In addition ammonium chloride or urea or both were given when salt restriction alone proved ineffectual. Under more strenuous treatment it might have been possible to eliminate edema at lower serum protein levels.

In similar studies Moore and Van Slyke (5) found edema in no case when the serum protein exceeded 5.5 per cent. Fahr Kerkhoff and Conklin (8), found proteins below 4.8 per cent in patients with nephritic edema.

#### *The relation of edema to serum protein fractions and oncotic pressure*

When the proteins were fractionated (see Figure 2), the results obtained were in accord with those of previous observers in showing that the deficiency involves chiefly, if not entirely, the albumin fraction. Globulin lay within normal limits or was slightly elevated. The relation of this hyperglobulinemia to the underlying infectious processes has already been mentioned. From molecular weight determinations



from 24.9 to 30.3 mm Hg. Although the albumin was reduced to the point at which edema is prone to occur in nephritis, the estimated oncotic pressure lay at all times above the level at which edema has been found to appear.

In the present study edema was present on every occasion when the serum albumin was below 2 per cent, but in only one instance when it exceeded 2.75 per cent. This exception is the case (83434) with heart block noted above. The observations with edema in the intermediate zone, 2.0 to 2.75 per cent, are composed chiefly of untreated or responsive cases. The dividing zone, in terms of oncotic pressure, seems to lie between 14 and 21 mm Hg with the same exception, case 83434 presenting edema, probably partly of cardiac origin, at an oncotic pressure of 22.9 mm.

These results are in keeping with those of other observers. Moore and Van Slyke (5) found edema only when serum albumin fell below 2.5 per cent. Fahr, Kerkhoff and Conklin (8) found that the serum oncotic pressure, determined by the method of Schade, was less than 17 mm Hg in patients with nephritic edema. Cope (6), using the osmometer of Verney, found oncotic pressures varying from 8 to 38 mm Hg in a group of edematous nephritics. The higher values were observed in the earlier stages of acute nephritis.

In the literature much weight has been attached to the albumin-globulin ratio. As it has been established that the concentrations of these two protein fractions in the serum are determined by entirely different factors and that the two fractions are probably functionally unrelated (1), the value of employing such a ratio is doubtful. It is not the proportion of albumin to globulin, but the absolute concentration of albumin, that is important in the edematous nephritides. A low albumin-globulin ratio due to reduction of albumin has a significance entirely different from that of an equally low ratio due to elevation of globulin.

#### *The causes of the serum albumin deficiency*

That the serum protein deficiency is not due simply to altered blood volume could be inferred from the fact that the two protein fractions are not equally affected. More direct evidence is found in studies of the serum volume. Linder, Lundsgaard, Van Slyke and Stillman (14)

made repeated determinations of the serum proteins and the serum volume (by the dye method) in a group of patients with chronic nephrosis or nephrotic glomerular nephritis. In no case was the serum volume appreciably above normal. Fluctuations of serum proteins and variations of edema were not attended by comparable changes of serum volume. Brown and Rountree (15) detected no abnormalities of serum volume in patients with renal edema. Darrow (16) noted a tendency to blood concentration during the edematous stages of nephritis in children. If the theory of Starling (12) offers a satisfactory explanation for the pathogenesis of edema, the blood should not share in the edema, which is due to the inability of the organism to retain fluid in the vascular bed.

It is generally believed, on the basis of indirect evidence of various kinds, that urinary albumin is derived directly from serum albumin which escapes from the blood circulating through the kidney, probably because of abnormal glomerular permeability. If this is the case albuminuria must be considered a serious cause of serum albumin wastage. A patient of average size losing as much as 16 grams of protein a day as did some of the cases in this series, would excrete all the albumin of his serum in the course of a week or two. Plasmapheresis experiments on dogs (17, 18) indicate that such a rate of removal seriously taxes the regenerative capacity. If the human capacity for protein regeneration is no greater than that of the dog, the urinary protein excretion of some patients in the present series must approach the limit of the regenerative powers of the body.

Nevertheless, as can be seen from Figure 3, the serum albumin deficit is not directly related to the degree of albuminuria. This lack of correlation holds not only for the group of patients, but also for those individuals who were observed over a sufficiently long period to permit valid comparisons to be made. Some of the patients with maximum albuminuria had relatively high serum proteins. Urine protein was determined by analyzing the urine for nitrogen before and after precipitation with trichloroacetic acid. In cases 35628, 61090, 34854, 95123, 83166, 50265, 80351 and 62246 serum albumin rose during the period of observation in spite of the fact that the degree of proteinuria remained practically unaltered. Therefore, although proteinuria is undoubtedly responsible, it is not solely responsible for the production of the serum protein deficit of the edematous nephritic.

It has been demonstrated by Frisch, Mendel and Peters (19) that serum protein deficit and edema can be produced in rats by the administration of diets containing insufficient amounts of protein. Furthermore, in earlier papers of this series (1, 20) it has been shown that serum albumin is regularly low in patients with malnutrition and may be restored to the normal level by the administration of diets with high protein and high calories. Ling (21) and Weech and Ling (22) have reported low serum albumin in Chinese famine victims. That nephri-

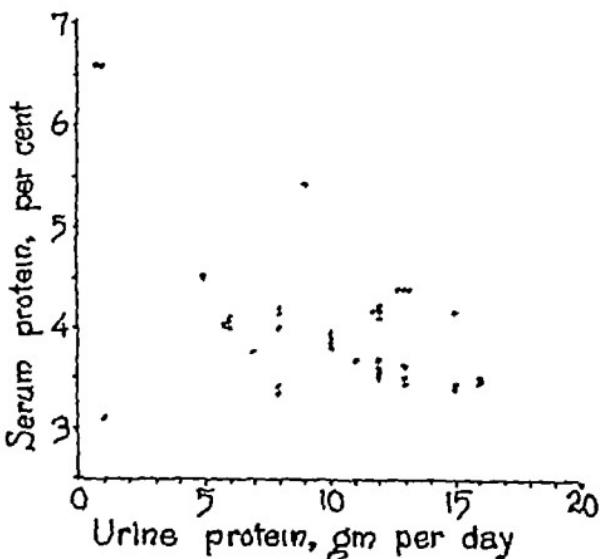


FIG 3 RELATION OF SERUM PROTEIN TO URINE PROTEIN

tis is a wasting disease has been clearly recognized by Bright and subsequent observers and has been emphasized by one of the authors (23). All these observations taken together suggest that malnutrition, or more specifically protein starvation, may be a factor in the production of the serum albumin deficiency in edematous nephritis.

An attempt has been made to estimate the state of nutrition in each case. This is difficult because the usual criteria of weight and appearance are of little value in the presence of edema. On the basis of other types of evidence, which are indicated in the following abstracts, it is possible to state that of the 21 patients 14 were unmistakably malnourished at the times when they presented edema and low serum proteins.

## SERUM PROTEINS IN NEPHRITIC EDema

TABLE I  
*Chronic glomerular nephritis*  
 (Case 61090, female, aged 29 when first seen)

Date*	Weight†	Urine protein stored‡	Urine protein	Serum			Diet §		NaCl grams per day	Remarks¶
				Breath metabolism	Protein	Globulin	per cent	grams per day		
1927				grams per day	grams per day	grams per day	per cent	per cent	per day	
July	6	95.0	1+	16	-20	3.20	1.08	2.12		
July	11	91.3	1+	12	44	3.48	1.79	1.69	54	Anorexia, nausea, and vomit-
July	23	91.1	1+	13	-55	3.48	1.51	1.97	49	Ammonium chloride
August	11	89.3	1+	12	595	2.93			1900	1
August	30	85.2	4+	5	188	3.62			1900	1 Urea
September	2	81.6	3+	6	91	3.91	2.04	1.87	2800	1 Urea and ammonium chlo-
September	13	55.9	2+	9	271	1.20	2.22	1.98	75	1 ride
September	21	49.3	0	13	233	4.37	2.04	2.33	90	1 Ammonium chloride, dires-
October	5	52.7	0	6	586	-25	4.02	2.27	1.75	105
October	20	56.4	0	8	189		1.18	2.29	1.89	110
October	25	57.5	0	7	161		4.34	2.59	1.75	118
October	31	58.4	0	5	136		4.47	2.51	1.96	120
December	7	(65.1)	#	1+		5.13	2.77	2.36	120	4000
1928										
February	6	(66.1)	0	4+		5.90	3.45	2.45	120	3500
June	17	(66.0)	0	4+		6.11	3.61	2.53	110	3000
September	23	(67.6)	0	3+		6.03	3.87	2.16	110	3000

TABLE I (continued)

Date *	Weight †	Edema	Urine protein	Protein stored ‡	Serum			Diet §		NaCl	Remarks ¶
					Brom-nitrobenzaldehyde	grams per day	Protein	Albumin	Globulin	grams per day	
1930	kilos				per cm <sup>3</sup>	per cm <sup>3</sup>	per cent	per cent	per cent	per day	
January 17	(66.6)	0	1	1	5.85	4.28	1.56	110	110	3000	
May 1930	(64.6)	0	1	0	6.57	4.03	2.54	110	110	3000	
January 29	(62.9)	0	3+		6.96	4.72	2.24	110	110	<3000	Diet reduced by patient
November 13	(64.3)	0	3+		5.95	3.91	2.04				Acute sore throat anorexia nausea and vomiting for 2 weeks
1931											Acute nephritis vomiting frequently, eating little
January 14	(70.9)	+	4+		4.29	2.30	1.99				
January 22	64.3	3+	4+		3.39	1.39	2.00				
January 27	62.6	+	8		3.79	1.64	2.15	55	1600	2	
February 6	57.4	0	9		4.63	2.78	1.85	110	3000	4	Digestive symptoms gone
February 11	59.1	0	4		5.00	3.25	1.75	110	3000		
February 23	(62.5)	0	3+		5.48	3.53	1.95	110	3000		
May 29	(68.2)	0	4+		5.31	3.64	1.67	110	3000		

\* Italics indicate visits to the outpatient clinic.

† Parentheses indicate that the patient was weighed with clothes.

‡ The protein storage is calculated from the total nitrogen retained since the date of the preceding observation.

§ Diet and remarks refer in each case to the period since the preceding date.

¶ Protein storage during urea periods is somewhat uncertain because of the inexplicable retention of nitrogen which occurs when urea is given (37).

case, the chief symptoms were referable to the digestive tract—anorexia, nausea and vomiting. These symptoms effectively thwarted attempts to give a high protein diet. Over a period of 5 months the average protein intake per day in no period exceeded 70 grams and for the first 2 months was only 50 grams. Meanwhile the edema stubbornly resisted treatment and the serum proteins persistently remained below 4 per cent. Six months after her first admission the digestive disturbances began to diminish and her diet gradually increased. Unfortunately the next blood examination was not made until 10 months later. By this time the proteins had risen to 4.53 per cent and she had only occasional orthostatic edema. The proteins in another 3 months had risen to 5.25 per cent with disappearance of the last vestiges of edema, although the proteinuria remained apparently unaltered. Further investigation of the effects of diet were frustrated by complete disappearance of the nephrotic syndrome with apparent restoration of normal kidney function. The important data in this case are presented in Table 3.

Case 95123 (see Table 4) was first seen in June 1928, when edema had persisted for a period of 6 years, under treatment consisting chiefly of protein restriction. At this time her excessive weight of 86.2 kilos was only partly referable to edema, chiefly to adiposity. The serum proteins were 4.07 per cent. Under treatment with a diet of 80 grams protein, 2000 calories, moderate salt restriction and urea, the edema disappeared and the proteins gradually rose to reach 5.89 per cent in November. About this time in behalf of her figure, at the request of the patient, the calories were reduced to 1500. The serum proteins had again dropped below 5 per cent by May, 1929, and after this failed to rise again even when protein and calories were apparently greatly increased. Subsequent questioning revealed the fact that the patient had not followed her diet prescriptions faithfully, fearing return of her obesity. The extent of her dietary lapses could not be ascertained. Shortly after the examination of May 22, 1930, which marked a new low point of 4.41 per cent for the serum proteins, she reduced her diet more drastically and, when edema shortly appeared, attempted vainly to check her rising weight by further restrictions. Although, when she returned for observation in November, she had subcutaneous edema and ascites her weight was not appreciably greater than it had been at

TABLE 3  
Nephrosis of unknown origin  
(Case 62246 female aged 30 when first seen)

Date*	Weight†	Edema‡	Urine protein	Protein stored†	Basal metabolism	Serum		Diet §		NaCl	Remarks¶
						grams per day	per cent	per cent	grams per day	Calories	
1927	<i>Nov.</i>	87.3	++			10	—23	—19	3.58	173	1.85
November 2							10	227	4.05	188	44
November 15	<i>Nov.</i>	79.9	++			10	1.53	3.91	2.06	1800	2
November 28		79.8	++				10		2.17	1900	2
November 5	<i>Dec.</i>	80.2	++			7	37.9	3.76	1.98	47	Ammonium chloride
December 14		78.2	++			12	113	3.59	1.85	55	Urea
1928									1.78	2000	2
February 7		78.0	++			11	4.10	1.85	1.74	50	Urea
February 16		74.2	+			11	176	3.86	75	1600	2
February 23		73.3	+			10	1.53	3.80	71	1900	2
March 7		74.5	+			10	228	3.94	1.60	234	Digestion normal, eating well
April 5		76.2	2+			4+	3.69	1.57	2.12	90	Digestion normal, eating well
1929										2000	2
February 20	<i>May 21</i>	(75.6) (74.8)	±	4+			4.53	3.40	1.13	90	Digestion normal, eating well
May 21			0	4+			—	5.25	3.04	2.21	Digestion normal, eating well
October 31		(79.2)	0	0			7.26	4.84	2.42	100	Came in without signs of renal disease, for anti-obesity diet
										2000+	

\* Italics indicate visits to the outpatient clinic.

† Parentheses indicate that the patient was weighed with clothes.

‡ The protein storage is calculated from the total nitrogen retained since the date of the preceding observation.

§ Diet and remarks refer in each case to the period since the preceding date.

|| Protein storage during urea periods is somewhat uncertain because of the inexplicable retention of nitrogen which occurs when urea is given (37).

TABLE I

## Chronic glomerular nephritis

(Case 95123, female, aged 25 when first seen)

Date *	Weight †	Edema ‡	Urine protein †	Protein stored †	Serum			Diet §		Remarks ¶
					Blood protein	Albumin	Globulin	Per cent	Grams per day	
1929					grams	per cent	per cent	per cent	grams per day	
June	26	(86.2)	2+	—	4.07	2.28	1.79	80	2000	After low protein diet
July	6	(82.2)	±	—					<5	Urea 20 grams daily
July	31	(78.6)	0	—					<5	Occasional urea
September	18	(73.8)	0	—					<5	Urea 10 grams daily
November	27	(66.3)	0	4+	4.66	2.70	1.96	80	2000	Diet reduced by patient to 1000 to 1200 calories shortly before this examination
1929					5.89	3.40	2.19			
March	20	(60.9)	0	4+					1500	
May	27	(59.5)	0	4+	+12	1.85	3.32	1.53	80	Actually took less than prescribed diet
August	1	(60.6)	±	4+		4.79	2.94	1.85	100	High diet taken for only a short period
December	17	(59.1)	0	8	+4	4.58	2.69	1.89	130	
1930									2800	
January	8	(60.1)	0	8						Diet again reduced
May	22	(60.1)	0	1+		4.41	2.32	2.09		Diet, especially calories, again low

TABLE 4 (continued)

Date*	Weight†	Edema	Urine protein‡	Protein stored§	Basal metabolism	Serum		Diet †		NaCl	Remarks¶
						grams per day	per cent	grams per day	per cent		
1930	61.7	3+	grams per day	-9	4.37	2.53	1.84				
November 13	61.7	3+	(61)	(16)	(5)	3.87	2.29	1.58	90	2500	In early summer reduced diet greatly. In September edema appeared and persisted.
November 21	57.3	+	8	(16)	4.52	2.09	2.43	90	2500	5	
November 26	57.6	0	6	(5)	3.89	2.33	1.56	120	2500	5	
December 4	(60.2)	±	4+								
December 17	(62.2)	±	13		3.95	2.36	1.59	110	2500	5	
December 29	62.5	+	19		3.68	1.90	1.78	110	2500	5	
1931											
January 6	61.3	0	16	14	-9	4.05	2.09	1.96	125	2500	3
January 10	59.9	0	8			4.15	2.32	1.83	125	2500	3
January 23	(65.2)	±	12			4.21	2.34	1.87	125	2500	5
February 11	(63.6)	±	6			4.03	2.45	1.58	120	2500	5
March 13	(63.2)	±	14			4.07	2.57	1.50	120	2500	5
April 15	(62.3)	±	12			4.17	2.44	1.73	120	2500	5
May 25	(62.5)	0	14			3.73	2.39	1.34	120	2500	5
June 30	(62.8)	0	12			4.41	2.67	1.74	120	2500	5

\* Italics indicate visits to the outpatient clinic.

† Parentheses indicate that the patient was weighed with clothes.

‡ The protein storage is calculated from the total nitrogen retained since the date of the preceding observation. Parentheses indicate that stools were not analyzed. Under these circumstances the stool nitrogen was estimated as 1.3 gram of nitrogen per day.

§ Diet and remarks refer in each case to the period since the preceding date.

|| Degree of proteinuria estimated from analyses of 24 hour specimens of urine brought to the outpatient department. In other instances urine collected in the hospital was analyzed.

the time of the previous observation. The serum proteins had fallen. With salt restriction, a high protein diet 2500 calories, and initial use of urea, the edema gradually disappeared when the protein rose above 4 per cent and the albumin above 23 per cent.

The evidence of protein depletion in these last cases though not unequivocal, is distinctly suggestive. If it is accepted, it is clear that malnutrition in the sense of protein wastage is not incompatible with obesity. In a limited sense this has already been proved by studies of the dietary treatment of obesity. Under such treatment there is invariably an initial negative nitrogen balance which does not parallel the loss of body weight. At first nitrogen loss is great in proportion to weight loss, later it diminishes or ceases while the weight continues to fall (28). The organism exhibits a certain, but not perfect, conservative tendency to utilize the indifferent fuel, fat, to spare the functionally important protein. The serum protein deficits which develop in malnutrition of other diseases and in famine victims clearly indicate that the organism to only a limited extent maintains serum albumin at the expense of tissue proteins. If, then, an obese person is afflicted with a proteinuria which continually drains away serum albumin and is at the same time, by reason of therapeutic restriction, indigestion or other functional disturbances, deprived of an adequate supply of protein, the serum albumin may rapidly fall through failure of the regenerative processes, while consumption of body fat, which is more directly dependent upon caloric needs, may proceed far more slowly.

In patients with true famine malnutrition von Hoesslin (29) and Weech and Ling (22) were unable, by feeding high calories in the form of carbohydrate and fat, without adequate protein, to eliminate edema, to increase weight or to restore the serum proteins to the normal level. The failure to cause weight increases is the more surprising because the basal metabolism, and presumably the caloric needs of malnourished subjects are subnormal. The body weight curves in Ling's cases, after the elimination of edema, at first paralleled closely the nitrogen retention. Somewhat comparable phenomena were observed in some of the nephritics of the present series.

Although 95123, in the earlier periods of study, after the elimination of edema, lost weight extremely slowly on diets of 1500 or 2000 calories when her weight had fallen and the serum proteins were low, her weight

remained almost constant on 3500 calories, although her basal metabolism was below normal, giving an estimated basal caloric requirement of only 1340 calories a day. Case 61090 gained weight only slowly on diets containing 3000 to 4000 calories.

#### *Basal metabolism*

Epstein (2, 30, 31), called attention to the frequent reduction of the basal metabolism in nephrosis, linking it with the lipemia, especially the hypercholesterolemia, and finding in it an indication for and an explanation of the beneficial effects of thyroid medication, which had been advocated by Eppinger. In Epstein's reported cases low basal metabolism occurs more consistently than it does in the literature in general. Nor have others been so uniformly successful with thyroid in the treatment of edema. In the present study basal metabolism was determined 32 times in 10 cases by means of the Roth Benedict apparatus. In only 6 instances, twice in 61090 with glomerular nephritis, 3 times in 34753 with amyloid nephrosis, and once in 62246 with idiopathic nephrosis, was the basal metabolism below -15 per cent. In 6 instances it was above 0, 4 times in the presence of edema. On the whole it seemed to be lowest during the most edematous periods and to rise as the edema disappeared and the general condition improved. However, in 80351, with idiopathic nephrosis, it was -2 and -7 on two occasions in the absence of edema, -4 in one of the edematous periods.

The reduction of basal metabolism that accompanies malnutrition has long been recognized (32) and is emphasized in Ling's (21) reports on famine edema. He found that edema in itself tended to reduce the metabolism (estimated in terms of body surface area) "because organism is diluted by a large mass of inert fluid." Malnutrition also had an effect. As nutrition improved the metabolism rose sharply at first with the disappearance of edema, more gradually later as nutrition improved. If nephritic edema is attended by malnutrition there is every reason to expect low basal metabolism, especially in the presence of edema.

#### *Therapeutic implications*

Epstein (33) was the first to attach any significance to the reduction of serum albumin as an indication for therapy. Assuming, logically,

the time of the previous observation. The serum proteins had fallen. With salt restriction, a high protein diet 2500 calories and initial use of urea, the edema gradually disappeared when the protein rose above 4 per cent and the albumin above 23 per cent.

The evidence of protein depletion in these last cases, though not unequivocal, is distinctly suggestive. If it is accepted, it is clear that malnutrition in the sense of protein wastage is not incompatible with obesity. In a limited sense this has already been proved by studies of the dietary treatment of obesity. Under such treatment there is invariably an initial negative nitrogen balance which does not parallel the loss of body weight. At first nitrogen loss is great in proportion to weight loss, later it diminishes or ceases while the weight continues to fall (28). The organism exhibits a certain, but not perfect, conservative tendency to utilize the indifferent fuel, fat to spare the functionally important protein. The serum protein deficits which develop in malnutrition of other diseases and in famine victims clearly indicate that the organism to only a limited extent maintains serum albumin at the expense of tissue proteins. If, then, an obese person is afflicted with a proteinuria which continually drains away serum albumin and is at the same time, by reason of therapeutic restriction, indigestion or other functional disturbances deprived of an adequate supply of protein, the serum albumin may rapidly fall through failure of the regenerative processes, while consumption of body fat, which is more directly dependent upon caloric needs, may proceed far more slowly.

In patients with true famine malnutrition von Hoesslin (29) and Weech and Ling (22) were unable, by feeding high calories in the form of carbohydrate and fat without adequate protein to eliminate edema, to increase weight or to restore the serum proteins to the normal level. The failure to cause weight increases is the more surprising because the basal metabolism, and presumably the caloric needs of malnourished subjects are subnormal. The body weight curves in Ling's cases, after the elimination of edema, at first paralleled closely the nitrogen retention. Somewhat comparable phenomena were observed in some of the nephritis of the present series.

Although 95123, in the earlier periods of study, after the elimination of edema, lost weight extremely slowly on diets of 1500 or 2000 calories, when her weight had fallen and the serum proteins were low, her weight

remained almost constant on 3500 calories although her basal metabolism was below normal, giving an estimated basal caloric requirement of only 1340 calories a day. Case 61090 gained weight only slowly on diets containing 3000 to 4000 calories.

#### *Basal metabolism*

Epstein (2, 30, 31), called attention to the frequent reduction of the basal metabolism in nephrosis, linking it with the lipemia, especially the hypercholesterolemia, and finding in it an indication for and an explanation of the beneficial effects of thyroid medication, which had been advocated by Eppinger. In Epstein's reported cases low basal metabolism occurs more consistently than it does in the literature in general. Nor have others been so uniformly successful with thyroid in the treatment of edema. In the present study basal metabolism was determined 32 times in 10 cases by means of the Roth Benedict apparatus. In only 6 instances twice in 61090 with glomerular nephritis, 3 times in 34753 with amyloid nephrosis, and once in 62246 with idiopathic nephrosis, was the basal metabolism below -15 per cent. In 6 instances it was above 0, 4 times in the presence of edema. On the whole it seemed to be lowest during the most edematous periods and to rise as the edema disappeared and the general condition improved. However, in 80351, with idiopathic nephrosis, it was -2 and -7 on two occasions in the absence of edema, -4 in one of the edematous periods.

The reduction of basal metabolism that accompanies malnutrition has long been recognized (32) and is emphasized in Ling's (21) reports on famine edema. He found that edema in itself tended to reduce the metabolism (estimated in terms of body surface area) "because the organism is diluted by a large mass of inert fluid." Malnutrition also had an effect. As nutrition improved the metabolism rose sharply at first with the disappearance of edema, more gradually later as nutrition improved. If nephritic edema is attended by malnutrition there is every reason to expect low basal metabolism, especially in the presence of edema.

#### *Therapeutic implications*

Epstein (33) was the first to attach any significance to the reduction of serum albumin as an indication for therapy. Assuming, logically,

that the low serum albumin was a direct result of leakage of such albumin into the urine, he advocated the administration of high protein diets to patients with nephrosis. Since then numerous others have reported favorable results from the use of generous protein diets. The data here presented offer further reasons for the use of such diets. How much protein should be given is as yet doubtful. In his original paper Epstein advocated 140 grams daily. It is, however, uncertain whether such large amounts can be utilized to advantage. The aim of dietary treatment is not to increase nitrogen catabolism, but to replace wastage and to provide extra protein for the restoration of previously depleted tissues. In the experience of the authors, except when there is infection or some other complicating condition, the protein catabolism of nephritic patients with edema is not abnormally high. In the cases studied during chronic periods it was possible to establish and maintain positive nitrogen balances, even in the face of severe albuminuria, with protein catabolism (estimated from urinary nonprotein nitrogen excretion) not greater and usually less than two thirds of a gram per kilo. In patients with malnutrition from other causes, it has been found that beyond a certain point, increasing increments of protein are used less and less efficiently for storage (20, 29). Although none of these patients were given more than 125 grams of protein daily, the capacity to store the excess protein with maximum efficiency was definitely surpassed.

In the present series of nephritic patients the amounts of protein taken seldom exceeded 125 grams and were usually much smaller. In most cases they were limited less by the physicians than by the appetites and digestions of the patients. When the patients were not afflicted by any complicating wasting disease and were able to consume over long periods enough protein to supply the endogenous metabolic needs plus enough to replace that lost in the urine and a further additional amount to restore previously depleted tissues, the serum proteins rose and edema disappeared. Although proteinuria continues unchanged such patients have been enabled to pursue normal active lives for periods of years. This is true not only of patients with apparently idiopathic nephrosis, but those with the nephrotic form of glomerular nephritis such as 61090 and 95123. Case 56577 shows that even a patient with amyloid nephrosis may become free from edema and

undergo a temporary remission if the underlying chronic infection becomes sufficiently arrested to permit restoration of the normal nutritive state

Whether this treatment has any definite effect in checking the progress of the disease and aiding repair of the renal lesion is doubtful, although it is not unreasonable to suppose that, by improving general health and nutrition, it may facilitate reparative processes. Two of the patients in this series, 80351 and 62246, appear to have recovered completely, the latter after an illness of almost three years duration.

Berglund and Scriver (34), Cowie, Jarvis and Cooperstock (27) and others claim that proteinuria is increased by high protein diets. Within the dietary limits employed in the present study no relation could be found between dietary and urinary protein.

Epstein (2, 33) advocates limitation of dietary fat in the treatment of nephrosis, because lipemia is so consistently encountered in this condition. Unless it can be proved that the lipemia is due to faulty fat metabolism, which seems unlikely (35), fat restriction does not seem to be clearly indicated. In order to secure maximum utilization of protein and to overcome malnutrition it is necessary to furnish adequate calories. This is almost impossible unless fat is given freely. There is no evidence in these studies that diets containing generous proportions of fat had any deleterious effects.

If the reductions of basal metabolism so frequently encountered are referable to edema and malnutrition, the administration of thyroid, which has been advocated by Epstein (2, 30), does not seem entirely logical. The low basal metabolism must be considered as a conservative process, and therefore one to be furthered and not combatted. Administration of thyroid to two of the cases of this series with low basal metabolism, 34753 and 62246, did not promote diuresis nor exert any other favorable effect, although it was pushed to the point where disagreeable symptoms appeared. Nor did these patients, as Epstein reports, exhibit any peculiar tolerance for the drug. It may be that the greater tolerance and beneficial effects reported by others are products of age and not disease. Most of these subjects have been children. It has been shown by Topper and Cohen (36) that in childhood thyroid does not produce symptoms of hyperthyroidism and may promote growth.

Although salt restriction and diuretic drugs, especially acidifying salts and urea, are useful measures in initiating diuresis, they should be considered only as temporary palliative measures and certainly should not be forced to the point where they interfere with appetite or digestion.

#### CONCLUSIONS

Reduction of serum proteins at the expense of the albumin fraction is not characteristic of idiopathic nephrosis, but is equally common in other types of nephritis with non-cardiac edema.

When the proteins, by reason of albumin deficiency fall below 5, but remain above 4 per cent, edema is likely to appear, but can usually be eliminated by proper therapeutic measures. When they fall below 4 per cent treatment is usually ineffectual.

Besides albuminuria, which permits direct loss of serum albumin into the urine, the chief cause of the serum protein deficiency appears to be depletion of the protein stores of the body.

#### PROTOCOLS

##### *Chronic glomerular nephritis*

*S5628* A Polish male, aged 43, developed generalized edema after a sore throat in August, 1924. He was admitted to the Hospital January 10, 1925, with slight anemia, general anasarca, blood pressure 122/78 phenolsulfon-phthalein excretion of 32 per cent, blood nonprotein nitrogen of 61 mgm per 100 cc, an inflamed throat and slight fever, urine containing blood and casts and from 8 to 19 grams of protein daily. On discharge he was free from edema, the blood nonprotein nitrogen was 30 mgm per 100 cc, and the urine contained no red blood cells, although the proteinuria had not diminished. The patient has not been seen again.

*61711* A boy, aged 5, developed generalized edema after a sore throat and otitis media, in November 1926. He was admitted to the hospital September 11, 1927, with general anasarca, secondary anemia, a blood pressure of 100/80, blood nonprotein nitrogen 22 mgm per 100 cc, and urine containing large amounts of protein without macroscopic or microscopic blood. He failed to respond to any therapeutic measures, had repeated upper respiratory infections (apparently due to the hemolytic streptococcus) and finally died January 2, 1928, of hemolytic streptococcus septicemia with peritonitis. Autopsy revealed in the kidneys glomerular nephritis, extensive tubular degeneration and focal lesions presumably connected with the septicemia.

*61050* An unmarried woman of 29, in March, 1927, developed generalized edema without any antecedent infection. She was admitted to the hospital

July 7 with general anasarca blood pressure of 160/108, phenolsulfonphthalein excretion of 15 per cent a blood nonprotein nitrogen of 77 mgm per 100 cc. and a urine containing 10 to 15 grams of protein daily, many casts, a few leucocytes but no red blood cells. After a period of resistance to treatment she developed a profuse diuresis and improved rapidly. At the time of discharge October 31 she was free from edema with a blood pressure of 124/96 blood nonprotein nitrogen of 29 without change in the urinary picture. The blood count gradually returned to normal and she improved steadily after her discharge. She was soon able to resume her occupation as cook and houseworker and felt entirely well until December 18, 1930, when she developed a severe upper respiratory infection. Edema recurred and she was finally admitted to the hospital January 21 with moderate general anasarca, but with normal blood pressure and a blood nonprotein nitrogen of 30. She improved rapidly under treatment, was discharged February 2 free from edema and was able to resume work a little later. The urine from the first observation to the present time, June 10, 1931 including the symptomless periods, has remained unchanged always containing large amounts of protein many casts and a few leucocytes, with only occasionally rare red blood cells.

34854 A Polish male, aged 29, was admitted to the hospital October 14, 1924. Two years earlier after an acute polyarthritis he developed general anasarca hematuria and lumbar pain. These symptoms persisted with exacerbations and remissions, the former usually accompanying sore throats. On admission he presented general anasarca secondary anemia, a blood pressure of 155/95 a phenolsulfonphthalein excretion of 45 per cent, blood nonprotein nitrogen of 43 mgm per 100 cc., and urine containing 10 to 15 grams of protein daily many casts, some leucocytes, and at intervals, numerous red blood cells. In addition the heart was enlarged, a loud systolic murmur was audible over the whole precordium, the spleen was considerably enlarged and hard, and he exhibited intermittent tenderness in both costovertebral angles, occasional arthritic manifestations and irregular fever. Under treatment after a considerable latent period, he developed diuresis and improved greatly. When discharged, on December 22, he was free from edema, the anemia had improved the blood pressure was 131/85, the blood nonprotein nitrogen 28 mgm per 100 cc. The urine was unchanged. Just after his discharge he caught cold developed pneumonia and was admitted to a Hartford Hospital with recurrence of all the nephritic symptoms. June 23, 1925 he was readmitted to the New Haven Hospital, where he remained until his death, October 8, 1925. Except for greater emaciation and anemia his condition was essentially the same as it had been on the previous admission. Under strict treatment he could be kept free from edema, blood pressure and blood nonprotein nitrogen remained normal. Irregular fever and intermittent temperature persisted, hematuria was more frequent and profuse and he suffered from attacks of sharp pain in the regions of the spleen.

and kidneys. September 19 he had a transient attack of unconsciousness followed by vomiting and severe headache. The spinal fluid was found to be bloody. October 8 he had a sudden convulsion and died a few hours later. Autopsy revealed a cerebral hemorrhage, a small, fresh vegetation on the mitral valve, old and fresh infarctions of spleen and kidneys. In the latter there were also extensive tubular degenerations, signs of chronic and acute glomerular nephritis, and lesions of a more acute, probably bacterial, diffuse focal nephritis.

56883 A male, in 1922, at the age of 16, developed nephritis with hematuria after an acute respiratory infection. The acute symptoms rapidly subsided, but he was left with persistent albuminuria and occasional periods of hematuria. In March, 1927 he spent 17 days in the hospital under observation. He was entirely free from symptoms, without edema or anemia. His blood pressure was 120/86, phenolsulfonphthalein excretion 56 per cent and his blood nonprotein nitrogen varied from 40 to 21 mgm per 100 cc. The urine contained 3 to 4 grams of protein daily, casts, and moderate numbers of leucocytes, but no red blood cells.

95123 A female, in 1922, at the age of 19, developed general anasarca after a sore throat. The edema persisted to a variable extent after subsidence of the acute attack. When she was first seen as an outpatient on June 22, 1928, she had moderate edema of the legs, no anemia, a blood pressure of 136/86, and a blood nonprotein nitrogen of 20 mgm per 100 cc. The urine contained 7 to 10 grams of protein daily, some casts and occasional leucocytes and red blood cells. After preliminary treatment with urea she became free from edema and remained so until the summer of 1930, when she reduced her diet for the sake of her figure. Shortly after this she developed anasarca and, November 12, 1930, was admitted to the hospital. Her edema disappeared with rest in bed and salt poor, high protein diet. After discharge she remained free from edema, at first with the aid of urea, later without. The blood pressure and blood nonprotein nitrogen remained normal throughout the urine unchanged.

83166 A male, aged 31, in February, 1930, began to suffer from pain in the back and the lower abdomen, relieved by urination, accompanied by edema of the ankles. The edema gradually increased and dyspnea on exertion and palpitation developed. In March the blood pressure was found elevated the lower trunk had become edematous and ascites had appeared. When admitted to the hospital, April 15, he presented signs of chronic tonsillar infection, rales and dulness at the bases of both lungs, ascites and edema of the lower extremities and the lower part of the trunk, a blood pressure of 140/90, 70 per cent phenolsulfonphthalein excretion, and a blood nonprotein nitrogen of 44 mgm per 100 cc. The urine contained 10 to 12 grams of protein daily, casts, a few leucocytes and rare red blood cells. He improved gradually under treatment until May 26, when he developed a sore throat and bronchopneumonia. With this the blood pressure rose the blood

nonprotein nitrogen increased to 60 mgm and vomiting began June 2, he went into convulsions. He was discharged against advice August 2. The blood pressure at this time was 150/100, the blood nonprotein nitrogen 76 mgm per 100 cc. The character of the urine had not changed. The patient died at home shortly after he left the hospital.

F M A female aged 14, in 1927, after a sore throat was discovered to have profuse albuminuria. After this she noticed that her eyelids became puffy at irregular intervals. When seen as an outpatient, December 16, 1930, she seemed well. There was no edema, no anemia, a blood pressure of 110/80, 50 per cent phenolsulfonphthalein excretion and a blood nonprotein nitrogen of 34 mgm per 100 cc. The urine contained about 3 grams of protein per day, rare casts, leucocytes and red blood cells. Her condition has remained unchanged until the present, June 22, 1931.

#### *Amyloid nephrosis*

94755 A male aged 19, was admitted to the hospital October 2, 1924 because of a severe diarrhea of 5 weeks' duration. This diarrhea persisted until death June 10, 1926, his feet became swollen and albumin was detected. He was admitted to the hospital again on June 14 and followed in the hospital or as an outpatient until death with acute peritonitis symptoms February 12, 1928. Throughout this period of study the diarrhea and edema persisted, the blood pressure and blood nonprotein nitrogen remained normal, the urine contained from 7 to 20 grams of protein daily, casts and leucocytes but no red blood cells. Autopsy revealed proliferative tuberculous enteritis and general amyloid disease with amyloid nephrosis.

56577 A female, in 1920, at the age of 12, developed an abscess of the os innominatum. After this she had repeated abscesses of the pelvic bones and one of the ribs. In January 1927, during the surgical treatment of one of these abscesses in this hospital, generalized anasarca appeared. The urine was found to contain large amounts of albumin, with casts and leucocytes but no red blood cells. The blood pressure and blood nonprotein nitrogen were normal. After a protracted illness the edema cleared and the abscess healed. The proteinuria diminished considerably. From August, 1928, she remained free from abscesses and symptoms of all kinds until December, 1930, when she had a recurrence of the pelvic osteomyelitis, which opened into the bladder. The edema recurred. Operation was unsuccessful and she died on April 11, 1931. The blood pressure was always normal, the blood nonprotein nitrogen rose only as a terminal event. Autopsy revealed osteomyelitis of the symphysis, pelvic peritonitis and amyloid nephrosis.

72481 A male developed pulmonary tuberculosis in 1927 at the age of 27. In September, 1928, after he had lost 26 lbs of weight, diarrhea and edema of the legs appeared. When he was admitted to the hospital January 13, 1929, besides evidences of advanced pulmonary tuberculosis, he had gen-



had, besides the previous findings, anemia, edema of the lower extremities and back, ascites, a blood pressure of 220/110, 30 per cent phenolsulfon phthalein excretion and a blood nonprotein nitrogen of 46 mgm per 100 cc. The urine contained 9 to 13 grams of protein, many leucocytes and some casts, but no red blood cells. Her condition became steadily worse and she died November 4.

#### *Cases of unknown origin*

**31100** Male aged 52 developed edema of the legs early in May, 1924. Ten months earlier he had contracted syphilis, for which he was treated with arsenical injections for 6 months. When he was admitted to the hospital on May 24 he had moderate edema of the lower extremities and back, a blood pressure of 140/80, 45 per cent phenolsulfonphthalein excretion, a blood nonprotein nitrogen of 32, and a positive blood Wassermann. The urine contained a large amount of protein, many casts and a few leucocytes and red blood cells. Under treatment in the hospital the edema diminished rapidly. However, it recurred when he left the hospital. When he was seen a year later he still had some orthostatic edema and the urine had not cleared, but his blood pressure and blood nonprotein nitrogen were normal.

**78222** A male, aged 17, in the middle of February, 1929, was seized with abdominal pain, vomiting, chills and temperature. Three days later albuminuria was discovered. The vomiting continued and edema gradually developed. When he entered the hospital March 27, he had general anasarca, anemia, a blood pressure of 118/72, a 15 per cent phenolsulfonphthalein excretion, and a blood nonprotein nitrogen of 72 mgm per 100 cc. The urine contained 10 to 16 grams of protein per day, many casts and a few leucocytes, but no red blood cells. Vomiting persisted after admission accompanied by headache. May 6 he developed diarrhea and increasing abdominal pain and tenderness with fever. On May 7 precordial pain appeared and May 10, harsh pleural friction on the right side. He died on May 10. Between May 6 and the day of death pneumococci, Group IV, were secured from 3 different blood cultures. An indefinite history of sore throat and rash just preceding the nephritis was obtained.

**83484.** A male, aged 68, developed pain in the joints of the upper extremities and in the back in May, 1929. November 19 he noticed edema of the lower extremities. In December he was found to have complete heart block and albuminuria was discovered. In March 1930, the pains extended to the lower extremities and the edema increased to involve the trunk. When seen at his home May 3, he had marked edema of all four extremities and the lower trunk, double hydrothorax, ascites, heart block, a blood pressure of 120/60, severe anemia, and a blood nonprotein nitrogen of 80 mgm per 100 cc. The urine contained 13 grams of protein per day, with few casts or cells. May 18 he was admitted to the hospital where he died on May 25.

29122 A male, aged 30, was admitted to the hospital February 2, 1921 because of edema which had appeared shortly before that without any antecedent infection. On admission he had general anasarca, a blood pressure of 105/65, 55 per cent phenolsulfonphthalein excretion and a blood nonprotein nitrogen of 33 mgm per 100 cc. His urine contained 10 to 15 grams of protein daily, with many casts but no red blood cells. The edema disappeared rapidly under treatment in the hospital.

80351 A male, aged 19, without known antecedent infection in March, 1928, developed edema, ascites, and albuminuria which persisted for four months. In March, 1929, he had a second similar attack which cleared up more rapidly. July 15, 1929, he was admitted to the hospital because of recurrence of albuminuria. There was no edema, the blood pressure was 124/64, phenolsulfonphthalein excretion 50 per cent, blood nonprotein nitrogen 25 mgm per 100 cc., and blood count normal. The urine remained clear while he was in the hospital. Early in September dizziness, anorexia, back pain and headache set in, associated with albuminuria. He was again admitted to the hospital, where the symptoms and urinary changes disappeared. His tonsils were removed because of a history of frequent sore throats. In November pain in the flanks recurred, accompanied by edema and profuse proteinuria. When he entered the hospital, November 19, he had general anasarca without anemia, hypertension or azotemia. His urine contained 6 to 8 grams of protein daily, with casts and leucocytes, but no red blood cells. Under treatment the edema gradually disappeared and the proteinuria diminished. By March, 1930, all signs of renal disease had disappeared. There has been no recurrence up to the present time (July, 1931). Industrial mercury poisoning may have been the etiological factor in this case.

62246 A female, aged 30, in June, 1927, without antecedent infection, developed edema of the feet and some breathlessness. These symptoms lasted only about 2 weeks, but recurred again in October, when albuminuria was discovered. When she was admitted to the hospital, November 1, 1927, she had moderate edema of the legs, thighs and lower back, slight anemia, a blood pressure of 105/75, 30 per cent phenolsulfonphthalein excretion and a blood nonprotein nitrogen of 32 mgm per 100 cc. The urine contained about 10 grams of protein daily, with occasional casts and leucocytes, but no red blood cells. The edema, although moderate, was extremely resistant to treatment. The patient suffered continuously from anorexia and digestive disturbances. However she improved gradually and, by October, 1928, the edema had disappeared although the proteinuria persisted unabated. She

is seen at infrequent intervals without evidence of change in the condition until in Mr., 1930, a distinct reduction of the proteinuria is noted. Shortly after this date the urine cleared completely. Examination in October, 1930 revealed no evidence of renal disease.

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